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Construction of a Pen-Scale Methane Collection System and Dietary Strategies for Methane Mitigation from Growing and Finishing Cattle

Thomas Winders
University of Nebraska - Lincoln, thomas.winders@huskers.unl.edu

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Thomas M. Winders

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Thomas M. Winders, M.S.

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Advisor: Galen E. Erickson

Methane production from ruminants has been researched for many years because it has a global warming potential 25x that of carbon dioxide, meaning that mitigating smaller amounts of methane can have a large environmental impact. Research has focused on individual animal methane measurements, but the literature lack in industry-scale measurements. For that reason, the methane barn was built to evaluate dietary strategies on pens of cattle rather than on individual animals. In order to test the methane barn capabilities, an experiment designed to produce differences in methane production was conducted. Cattle received the same growing diet, at either ad-libitum access to feed or restricted access to feed (75% of the ad-libitum intake). Methane (g/d) was lower for the limit-fed cattle, which gave us confidence that the methane barn was working correctly. The second trial evaluated the effects of corn oil on methane production and animal performance. Corn oil reduced methane production (g/d, k/kg ADG) in finishing cattle and improved performance. Feed additives that alter the rumen environment as a methane mitigation strategy have shown some promise. Biochar was evaluated using in vitro and in vivo methods to determine gas production as well as digestibility characteristics associated with feeding it. In vitro results showed that as biochar

inclusion increased, total gas and methane increased. When tested in vivo, biochar inclusion reduced methane and carbon dioxide production compared to control. OM digestibility and NDF digestibility were greatest for 0.8% inclusion of biochar, while 0 and 3% were not different.

Key words: beef cattle, biochar, corn oil, intake, methane mitigation

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"Life means to me living in the fullest sense, having a healthy and fit body and an active and enquiring mind. It means feeling all the emotions like love, joy, anger, frustration, sorrow, and pain. It means laughter and crying, enjoying the company of friends, and having the tolerance to put up with jerks. Accepting authority, even when the commands are silly, and being able to accept criticism from others. But most of all, life is for giving. Giving of self to others in service or love, for it is in giving of oneself that I can become part of the life of God."

Bert Winders, 1982

"Strap yourself to a tree with roots" – Bob Dylan

"I'm gonna be a happy idiot" – Jackson Browne

"To be a rock and not to roll" – Led Zeppelin

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Introduction

Methane (CH_4) production by cattle is an energetic loss to the animal as well as a concern for the environment because of its global warming potential as a potent greenhouse gas. Ruminants have been estimated to contribute around 16% of total methane production on a global scale, and are considered one of the main sources of anthropogenic methane production (Eugene et al., 2008; Mathison et al., 1998, Moss et al., 1992). However, in the US, enteric fermentation from cattle account for only 2.7% of carbon dioxide equivalents, and agriculture as a whole is only 8.6% (US EPA, 2016). The eructation of methane results in a 6% loss of ingested energy to the animal, so it would be beneficial for production purposes to retain this energy as well as being beneficial to the environment (Johnson and Johnson, 1995). The discovery of the process of bacterial methane oxidation happened in 1905-1906 by Kaserer (1905) and Sohngen (1906). The environmental concerns paired with the energetic loss to the animal are the driving forces behind research on methane production dating back to 1949, when Hutchinson et al., (1949) first estimated methane production by large herbivores. Since then, methane has been studied for various reasons, with the central focus being mitigation strategies. One of the main strategies for manipulating methane production in cattle has been through dietary changes.

In order to mitigate methane production in ruminants, we must be able to accurately measure and quantify methane. The "gold-standard" form of measuring methane has been through the use of calorimetry chambers which are good for measuring

individual animal emissions but fail to replicate modern production settings. Enteric fermentation, methane collections, and mitigation strategies will be discussed in detail in the review of literature.

CHAPTER I. Review of Literature

Methane

The global annual contribution of atmospheric methane is 500-600 Tg which comes from microbial processes that take place in the environment such as in wetlands, flooded rice fields, burning fossil fuels, biomass burning, ruminants, termites, landfills, oceans, gas hydrates, and sewage treatment. Of these microbial contributors to global methane production, wetlands contribute 23% of the global budget which is the most. The second largest contributor the global methane budget is fossil fuels at 18%. Third is the ruminant animal, contributing 17% of global methane production, and 28% of anthropogenic (under human control) methane, which is a main reason why there has been an increasing focus on in mitigating ruminant methane production (Beauchemin et al., 2008; Conrad et al., 2009). In the US, cattle emissions are lower, as only 34% of agriculture's contribution to greenhouse gas emissions comes from enteric fermentation, and agriculture accounts for only 9% of all emissions (US EPA, 2016).

Ruminant animals eructate methane through a natural pathways during microbial fermentation in the rumen. This biological process is a huge benefit to a growing human population that is expected to reach or exceed 9 billion people by 2050 (Capper, 2012) because ruminants are able to turn carbohydrates that humans can't digest (cellulose) into meat and milk for human use. Digestion of cellulose is possible because of a microbial population present in the rumen capable of breaking down fiber into volatile fatty acids. A downside to this process is the environmental concerns associated with methane

production as a waste product of ruminal fermentation. Carbon-containing compounds (referred to hereon as 'carbons') leaving the rumen present a potential energetic loss because carbons can be used for energy, but the process of eructating methane is necessary otherwise gas would continue to build up in the rumen and lead to rumenstasis. With global temperatures increasing, and methane being a potent greenhouse gas, many governments have implemented policies searching for ways to reduce anthropogenic methane production (Beauchemin et al., 2007). Methane is 25x more potent of a greenhouse gas than carbon dioxide and is being added to the atmosphere at a rate of around 1% more methane a year due to a production to degradation ratio imbalance (Trotsenko and Murrell 2008). Not only is methane production an environmental concern, but also a production efficiency concern due to loss of ingested energy ranging from 2 to 12 %, typically around 6% (Johnson and Johnson, 1995). The gross energy (GE) of methane is 0.88 MJ/mol, which is the same as the GE of acetate. The energy lost as methane would be beneficial for the animal to retain for production purposes (Bodas et al., 2012).

Carbon dioxide is present in the rumen and is an often used substrate for methane production. Carbon dioxide is a by-product of VFA production resulting from microbial fermentation. Carbon dioxide is closely related to animal metabolism and energy expenditure more so than ruminal fermentation. According to Peterson et al. (2008), animal carbon dioxide production is dependant on body mass and feeding level and is closely related to the respiratory quotient. Cellular respiration and basal metabolic rate play a large role in carbon dioxide production, and is closely related to energy

expenditure. Peters et al. (2010) illustrate that cattle on a grain-based diet will have greater carbon footprints due to an increase in metabolic rate (more energy in the diet leads to more growth) than cattle on forage-based diets.

Enteric Fermentation

Enteric fermentation is the biological process that breaks down feeds primarily into volatile fatty acids (VFA) in the rumen through the use of microbes. Volatile fatty acids are the main energy source for the animal once absorbed across the rumen wall. The main by-products of VFA production in the rumen are carbon dioxide, methane, and hydrogen. These are formed by a large number of different microbial species. Bacteria are the most prominent in rumen fluid ($10^{10} – 10^{12}$ ml^{-1}), protozoa are second ($10^{5} – 10^{6}$ ml^{-1}) and fungi are third ($10^{4} – 10^{5}$ ml^{-1}) (Patra et al., 2011). Methanogens belong to a different phylum (*Euryarchaeota*) but are in the same domain (Archaea) and are present at concentration of $10^{8} – 10^{10}$ ml^{-1} (Hook et al., 2010).

Methanogens

Methanogens are present in a wide range of harsh anaerobic environments such as wetlands, oceans and rice fields, but this review will focus on methanogens in the rumen. Of the 113 methanogen species identified in nature, only seven are commonly present in the rumen. These seven species are *Methanobacterium formicicum*, *Methanobacterium bryantii*, *Methanobrevibacter ruminantium*, *Methanobrevibacter millerae*, *Methanobrevibacter olleyae*, *Methanomicrobium mobile*, and *Methanoculleus olentangyi* (Patra et al., 2011; Janssen and Kirs, 2008). Methanogens from the family

Methanobacteriaceae are the most prominent in the rumen, consisting of 30 – 90% of the archaea. Methanogens from the order of *Methanomicrobiales* are second most abundant, consisting of 0 – 54% of total methanogens in the rumen, while members of the order *Methanosarcinales* are least abundant (2 – 3%) (Janssen and Kirs, 2008). *Methanobrevibacter ruminantium* is a non-motile, Gram-positive species that is the most abundant in the rumen of all methanogenic species (10^6 – 10^8 ml^{-1}). Methanogens thrive in an environment with a pH range between 6 and 8 and can only grow in environments with redox potentials lower than -300 mV (Mathison 1998).

Methanogens are a very diverse species with many differences between them, but one consistent aspect is the lack of usable substrates. The substrates available to methanogens are carbon dioxide, acetate, formate, and compounds containing a methyl group such as methanol, methylamines, and methyl-sulfides (Leiber, 2014). Methanogens cannot utilize most organic compounds, such as carbohydrates or alcohols directly, as they must wait for other microbes to ferment these products into usable substrates for methanogenesis. It is unclear why methanogens cannot utilize most organic compounds and therefore act independent of other microbes (Liu et al., 2008). Almost all methanogens use H_2 and formate as their energy sources for growth and reproduction, which allows for the electrons from either substrate to reduce carbon dioxide present from VFA production into methane in the rumen. A few species of methanogens (*Methanosarcina)* are able to use acetate for growth, in turn making carbon dioxide and methane, but this is not a common process in the rumen (Janssen and Kirs, 2008). From these two processes of methanogenic growth come two different types of methanogens: slow-growing methanogens (130 h) that dissimilate acetate into carbon dioxide and

methane, and fast-growing methanogens (4 to 12 h) that reduce carbon dioxide with H_2 and formate (Patra et al., 2011). The fast-growing methanogens are far more prevalent in the rumen due to rumen particle passage rates, which hinders the slow-growing methanogen process because they require more time to use acetate for their growth. In most environments other than the rumen, slow-growth methanogenesis is predominant (Weimer, 1998).

Ruminant research has targeted mitigating methane through microbial manipulation, or reducing methanogenesis, but if all methanogens are removed, the H_2 produced during fermentation would need an alternative route. Many of the alternatives (feeding more lipid, nitrates, and sulfates) can have negative digestive or health problems for the animal. One promising route for using the electrons is acetogenesis, although they have a poor affinity for H_2 electrons relative to methanogens. Certain environments allow for acetate production to remain competitive with methanogenesis such as in the human colon or in the hindguts of termites. The phenomena as to why they are able to compete more in those environments compared to the rumen is still largely unknown. (Weimer, 1998).

Methane production in the rumen

Methane production in the rumen is a result of organic matter fermentation by the rumen microbes in an anaerobic environment. Volatile fatty acids are an end product of fermentation by rumen microbes and are the main energy source for the animal, accounting for 65% of the glucose used by the animal when fed a high-concentrate diet, with the other 35% coming from deamination of amino acids. Methane production is a

by-product of VFA production, except when propionate is being produced. Propionate is a hydrogen sink in the rumen and therefore accepts hydrogens, whereas acetate and butyrate release hydrogens that need to be expelled, with one form of removal being methane. Methanogenesis is the process of forming methane, and can be done through four pathways: hydrogenotrophic, aceticlastic, methylotrophic, and the methyl reduction pathway (Welander and Metcalf, 2005). In order for methanogens to function, there are different cofactors that must be present, with a few of the major ones being methanofuran (MFR), tetrahydromethanopterin (H_4MPT), coenzyme M (CoM), coenzyme B (CoB), and coenzyme F_{420} (Liu 2008).

The hydrogenotrophic pathway (Fig. 1A) is a very common carbon dioxide reduction pathway resulting in methane production by reducing carbon dioxide (Welander and Metcalf, 2005). When carbon dioxide is the substrate, it can be reduced to methane using H_2 as the primary electron donor, but can also use formate. The formate process requires four molecules of formate to be oxidized to carbon dioxide by formate dehydrogenase (Fdh), and then one molecule of carbon dioxide can be reduced to methane. The aceticlastic pathway (Fig. 1C) dismutates acetate, which then forms acetyl-CoA. The carbonyl group left in this process is oxidized to carbon dioxide and the methyl group forms into tetrahydrosarcinapterin (H_4SPT) and is then reduced to methane. The methylotrophic pathway (Fig. 1B) uses C-1 structures, like methanol and methylamines, and forms carbon dioxide and methane from them. This process only works if one molecule of substrate is oxidized into the reducing equivalents needed to reduce three molecules of methane. The methyl reduction pathway uses H_2 just like the

hydrogenotrophic pathway, but reduces methanol to methane after coenzyme M (CoM) receives the methyl group (Welander and Metcalf 2005).

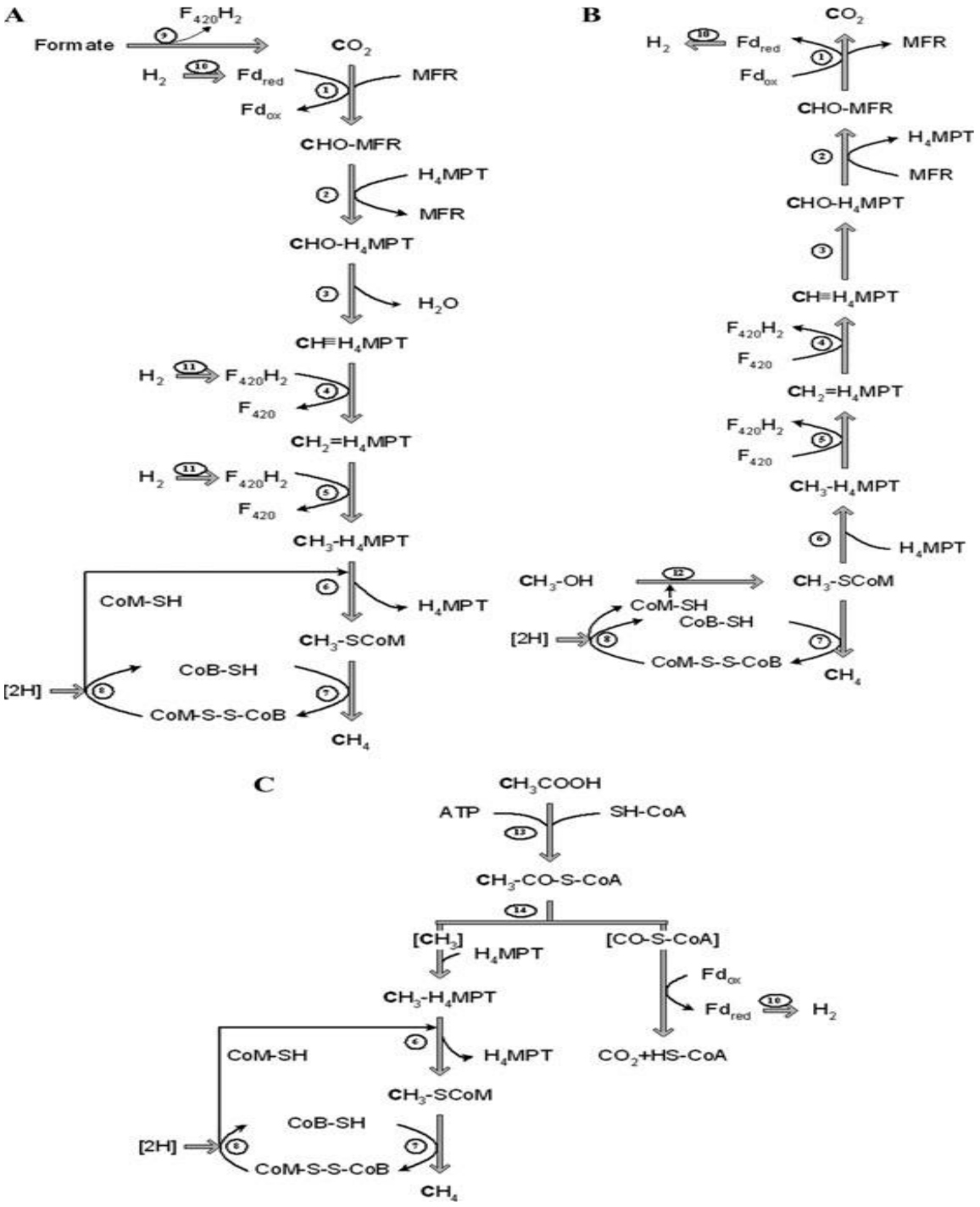

Figure 1. Three pathways utilizing three different substrates. A is utilizing CO_2 through formate, B is utilizing methyl groups, and C is utilizing acetate. This demonstrates the varying ways methane can be produced based on initial substrate (Liu et al., 2008).

Methanogens are important to the rumen environment because they regulate overall fermentation by removing H_2 gas. Reduction of carbon dioxide to methane is done primarily by using H_2 as stated previously in this review. The H_2 primarily comes from the fermentation of glucose to pyruvate in the rumen. Rumen bacteria have been classified based off of H_2 production in three groups: bacteria that produce propionate, butyrate, ethanol and lactate without producing H_2, acetate producing bacteria that yield H_2, and methanogens that consume H_2. The second group is dependent on the third group for fermentation to carry on efficiently, meaning propionate production is a major pathway for the rumen to dispose of some reducing power, resulting in an inverse relationship between propionate and methane. The recycling of NAD^+ in the rumen plays an important role in H_2 production because methanogenesis needs an electron acceptor, therefore turning NADH into NAD^+ and yielding H_2. The bacteria capable of oxidizing reduced electron carriers (NADH or ferrodoxin) to produce H_2 are the acetate producing bacteria (Bodas et al., 2012). Keeping the H_2 levels low is essential in the rumen in order to promote the growth of other microbes that aid in rumen fermentation. This process is sometimes referred to as interspecies hydrogen transfer, and refers to the interaction between H_2-producing species and H_2-utilizing species (methanogens).

When the methanogens remove H_2 and keep it from building up in the rumen, it promotes H_2-producing species such as *Ruminococcus albus*, resulting in more energy producing pathways. When *Ruminococcus albus* is present alongside methanogens, the end products of fermentation are acetate and methane. When *Ruminococcus albus* is in a methanogen-free environment, the end products of fermentation are acetate, ethanol, H_2, and carbon dioxide. This means that methanogens aid in energy yielding fermentation rather than being a waste of energy by keeping H_2 levels low and as a result, increase ATP production by H_2-producers (Church, 1998).

Cattle's estimated contribution to global methane production

Cattle have come under scrutiny due to their environmental impact resulting from enteric methane production. Methane loss by the animal is perceived as a negative for the environment as well as an energetic loss to the animal. Agriculture is the biggest contributor of non- carbon dioxide emissions, accounting for 58%, with enteric fermentation accounting for 33% of that 58% (EPA, 2012). Total Agriculture methane emissions have been estimated to be as high as 50-60%, with most coming from livestock fermentation (Hook et al., 2010). Total GHG emissions from agriculture have been estimated at 37% by Tubiello et al. (2013). The same authors estimated beef cattle contributed 56% of the total enteric fermentation, with dairy cattle being second at 19% of total agriculture contributions. A lower estimate showed that livestock contribute 9-11% of total anthropogenic GHG emissions (Pickering et al., 2013).

Cattle are the largest agricultural contributors to non- carbon dioxide emissions, which is a main reason for mitigation needs (EPA, 2012). The global methane budget is

estimated at 500-600 Tg methane per year, with ruminant livestock making up roughly 17% of this total (Conrad et al., 2009). Capper et al. (2011) estimated livestock to contribute 18% of total GHG on a global scale. Beauchemin et al. (2008) estimated ruminant livestock to contribute 28% of anthropomorphic methane emissions globally, at 80 million tonnes, but have been estimated to contribute as much as 86 million tonnes (Hook et al., 2010). Atmospheric methane can be broken down into natural production, and anthropogenic production. Mathison et al. (1998) showed the anthropogenic methane to contribute 70% of the total atmospheric methane while natural sources were 30%. Johnson and Johnson (1995) estimated livestock to contribute 25% of anthropogenic methane and 17% of total global methane. All of these estimates are quite variable and could be due to the boundary of the systems used to estimate methane as well as that there is no consensus values for livestock emissions. The varying results lead into why further research is being done on methane emissions from livestock. Although GHG numbers have been rising, agriculture has done a good job of becoming more efficient in terms of production of GHG per unit of production, which has declined over the past 50 years. In 1994 it took 25.6 million cows to produce 53 billion kg of milk, compared to 9.2 million cows and 84.2 billion kg of milk in 2007. Beef production has shown a 16% decrease in CO_2-eq per billion kg of beef produced in 2007 compared to 1977 (Capper 2009, 2011).

Collection methods

Estimating global methane production is only as useful as the methods used to quantify methane production. There are a variety of different ways used to quantify

methane production, with varying reasons for choosing one method over another. Some of these reasons would be resources available such as number of animals or space, ease of operating, expense of needed equipment, and end goal from doing the experiment, i.e. do you want the results to represent a production setting or done so on an individual animal basis?

Respiration Calorimetry

Methane work has been done using many different methods, but the "gold standard" is using a respiration chamber (RC). These chambers are also referred to as respiration calorimeters and provide an accurate measurement method for gaseous emissions. Animals are usually subject to 1-3 day collection periods and often utilize the ability for continuous or frequent gas sampling. The frequent sampling can account for the fluxes in gas emissions as a result from belching versus times when the animal is just breathing without a rumen release of gas, as well as account for diurnal variation. Respiration calorimeters can be categorized as either open-circuit or closed-circuit systems. Open-circuit calorimetry is more commonly used today and is when air is continuously circulated through the apparatus. Open-circuit systems must take into account the flow rates of incoming and exiting air and analyze the airstream prior to entering and as it leaves the apparatus. There are different types of open-circuit calorimeters based upon the animal confinement during collections. A closed-circuit system is when the air that enters the apparatus is recycled back to the animal after it has been analyzed and uses absorbents to remove water vapor and carbon dioxide from the air (Judy, 2017). A potential flaw that can occur from measuring methane is inability to

collect hindgut and manure methane production. However, Murray at al. (1976) has shown that 89% of methane production from a ruminant occurs from ruminal fermentation and is exhaled through the mouth and nose, leaving only negligible amounts to come from hindgut fermentation. Up to 95% of gases produced by enteric fermentation in the rumen are eructated, leaving only 5% to be expelled through regular breath. Eighty nine percent of the hindgut enteric fermentation gases are eructated as well, with only 11% of the 89% exiting in manure or flatulence. The concentration of methane in breath is low when the breath comes from the lungs, and is high when it comes in the form of a belch from the rumen (Koch et al., 2009). Methane is largely expelled through intermittent burps or eructations that are high in methane, while still exhaling basal levels consistently through normal respiration, so second by second measurements in an RC are not commonly used. Most of the time volume of methane is measured every 5-6 minutes, which can average out the belches to get consistent results. The gases that are typically measured in an RC setting would be carbon dioxide, methane, and O_2 and are measured in an ambient air line as well as an exhaust line from the chamber. End results quantify total volume of carbon dioxide and methane produced as well as O_2 consumed. Oxygen is typically used when trying to calculate energetics.

A major issue with this method would be the stress placed on the animal due to an abrupt environment change. When this happens, the animal could eat less feed than usual, as well as change feeding, drinking, and resting behavior. A drop in feed intake is a concern for gas collections because intake is big contributor to amount of gas emitted, so it is considered a limitation to this collection method (Madsen et al., 2010). Another limitation is the expense associated with the calorimetry chambers and subsequent gas

analyzers needed to measure gas emissions. The RC method also puts a limit on the number of animals that can be measured due to expense and labor limitations. A respiration chamber fails to represent an actual production setting, due to an unnatural collection setting which is necessary to capture all or most gas emissions, but doesn't reflect animals in their typical environment, whether that is on pasture/grazing setting or a confined setting. Providing the diet in the chamber also eliminates the animals' ability to select its intake, and leaves it with the only options of eating or sorting instead of selecting (Pickering et al., 2013). Lastly, a limitation regarding animal training needs to be considered as the animals need to be halter-broke, and introduced to the chamber if it is an open-circuit neck and head chamber (Johnson et al., 1994). Considering all the positives and negatives, open or closed-circuit calorimetry is the only method that can quantify gas production by capturing most or all of gas emissions, therefore making it the gold-standard for methane measurement methods (Pickering et al., 2013).

Sulfur hexafluoride tracer method

Sulfur hexafluoride (SF_6) is another commonly used method for methane collections. The SF_6 method was first described by Johnson et al. (1994) and has been adapted by many others since then due to some of the advantages provided. This method has been used widely in grazing work because the animals are able to move about freely and have the ability to select their intake, which is more applicable to production grazing settings. Sulfur hexaflouride is a gas that is used as a tracer to measure methane production by placing a known amount of SF_6 in a permeable tube inside the rumen. The permeable tube releases the SF_6 at a constant, known rate and is expelled at a constant

rate through respiration and eructation, the same way that methane is expelled. The idea behind the method is that methane and SF_6 will be mixed and diluted the exact same way by the time they exit the mouth and nostrils. This assumption is the key to the SF_6 tracer method. The permeable tube is filled with SF_6 and placed in a rumen temperature (39°C) water bath for multiple days or weeks to establish what the flow rate is once the gas release has stabilized. Once placed in the rumen, the animal will be equipped with a canister with tubing running from the canister to the mouth/nose area. Once collections take place, the capillary tubing will draw samples into the canister where it will be stored until the collection period is over. A typical collection period is 2-6 hours long, and replicated multiple times over a 24 period, and can be repeated over 5-10 days, or until the SF_6 release is unstable. The gas in the canister is then analyzed for SF_6 and methane using gas chromatography (GC) after being pressurized with nitrogen gas (Johnson et al., 1994; Pickering et al., 2013; McGinn et al., 2006).

Johnson et al. (1994) validated the SF_6 collection technique by comparing it against open-circuit, indirect calorimetry data on a liter per hour basis. No difference in collection method was observed on a heifer tested in both environments. Similar results were repeated with heifers and steers, on high roughage and high concentrate diets with no differences being detected, therefore validating the SF_6 collection method. The results showed that the SF_6 method estimates were numerically 7% lower than the values collected in the respiration chamber. Another validity test was done with 10 sheep showed that the SF_6 method collected values that were 5% lower than respiration chamber emissions results. Others have had trouble replicating these results using the tracer method, with error coming from having to transport the gases from their collection

site to the analysis site (Ulyatt et al., 1999). A study showing 46 comparisons between cattle collected in a chamber vs. the SF_6 method being fed high grain or high forage diets showed a 4% numerical difference ($P > 0.40$) that is in line with the results from Johnson et al. (1994) and Ulyatt et al. (1999).

Pinares-Patino et al. (2003) did a review on the reliability of the tracer method versus the calorimetry method. First, the permeation rates (PR) of the tubes that hold SF_6 in the rumen can vary and if the calibration of the tubes happens well before the trial, it can lead to a lower release rate of SF_6 and therefore give inconsistent results, with PR accounting for 6 to 21% of the total methane variability in one study. Pre-trial calibration of the tubes should occur no earlier than 4-6 weeks before the trial begins. There is a linear relationship between PR and methane production, meaning that the more SF_6 released from the permeation tube, the greater the estimate of methane becomes, which can inflate the results of actual methane production (Pinares-Patino and Clark, 2008). Animal-to-animal variation is a concern for this method because it tends have a greater range than it does using respiration chambers due to the method relying on a tracer rather than actual methane collections as seen in a respiration calorimeter. Pineras-Patino et al. (2003) showed that 54-70% of the variation in methane emissions is due to this variation. These same authors showed that animal variation in the tracer method versus the chamber method was 13.8% vs. 5.5% respectively. Grainger et al. (2007) showed this comparison to be 19.6% vs. 17.8% for tracer vs. chambers. However, Oss et al. (2016) showed a lower animal-to-animal variation between SF_6 (6.8%) and chambers (7.5%). If indeed variation is greater using the tracer method, more animals will be needed in order to detect differences in gas production, although the previously cited work is conflicting.

Also, SF_6 is a potent greenhouse gas and has been banned in several countries, making it off limits for research purposes (Madsen et al., 2010).

An advantage to the SF_6 method would be the ability for the animal to be out on pasture while collecting methane, meaning the animals' environment is not changing. This is an advantage over the RC chamber method because it eliminates the stressors associated with changing environment, and should keep the animals eating, drinking and resting behavior the same once acclimated to wearing the collar. Another advantage is the low cost relative to using respiration chambers and can be done in a herd setting. Overall, SF_6 used as a tracer has been shown to underestimate methane in some scenarios (McGinn et al., 2006) and overestimate it in others (Munoz et al., 2012), with a tendency for greater animal variation compared to chamber collections (Oss et al., 2016). This method remains one of the most common ways to quantify methane production today even though it isn't a perfect system.

Using carbon dioxide as an internal marker for methane

Madsen et al. (2010) developed a method based on carbon dioxide's relationship with heat production with the goal of meeting some of the weaknesses of the other methods. This method is simple and inexpensive relative to the methods discussed above which has made it a common way to estimate emissions without having to use a tracer gas or a respiration chamber. This method is based on heat production and carbon dioxide excretion by the animal, using data from calorimetry data or building ventilation data confirming the close relationship between carbon dioxide and heat production. Chwalibog et al. (1991) calculates heat production by subtracting products derived from

energy (milk, gain) from the metabolizable energy ingested. Animal ventilation data uses heat producing units (HPU) to establish a relationship between carbon dioxide and heat production from animals (Madsen et al., 2010). Taking simultaneous collections of methane and carbon dioxide measured in breath, as well as total estimated carbon dioxide production to estimate methane production served as the baseline for this methodology (Hellwing et al., 2013).

Hegarty et al. (2013) explained that carbon dioxide is much less variable than enteric methane, and can therefore be used as an internal marker to estimate daily methane production as a means of scaling up short-term emission collections. Expressing the short term collections as daily production must be done so with a scaling-up coefficient used by Garnsworthy et al. (2012) in order to limit bias in final values. Since carbon dioxide is less episodic than methane, emissions can be portrayed as a ratio of CO_2:CH_4 allowing for varying methane levels to be detected with a somewhat stable production of carbon dioxide. Both carbon dioxide and methane have a positive correlation with feed intake, so monitoring intake is crucial for estimating the amount of carbon dioxide emitted (Pinares-Patino et al., 2007). Using this ratio may be the most accurate way to express the efficiency of microbial fermentation because it describes the carbons that are lost that weren't converted to carbon dioxide. This information could be useful in determining which feeds are more efficient, resulting in less methane production (Madsen et al., 2010).

This ratio measurement has been shown to be more repeatable than the tracer method in a study done by Lassen et al. (2012) which could be useful as a genetic selection tool to select for lower methane-producing cattle. These authors determined

dairy cattle to have a repeatability of 0.37 and 0.33 for Holsteins and Jerseys, respectively, when using the ratio to determine methane production. Lassen et al. (2010) determined the CO_2:CH_4 ratio based on intake and body weight to be repeatable at 0.35 and 0.37 respectively, indicating that there may be strong enough repeatability to use genetic evaluation to make robust estimates of methane production across a large number of animals (Hegarty et al., 2013). This does not mean however that the Madsen et al. (2010) method is more accurate than actual full day measurements. Hellwing et al. (2013) did a comparison of indirect calorimetry chambers to the Madsen et al. (2010) equation using 157 observations of lactating dairy cows across 30 different treatments. The results showed that although the Madsen et al. (2010) equation explained 55% of the methane production variation, it consistently underestimated methane production by an average of 17%. The same authors also showed that methane production, energy corrected milk, carbon dioxide production, and body weight explain almost all of the differing results in methane emissions, with carbon dioxide production being the main estimate flaw. Using carbon dioxide as an internal marker is a common method for methane production due to ease, flexibility, expense, and large number of observations possible, but there is room for improvement and more work should be done in order to get more accurate predictions of total methane emissions.

Short-term methane measurements

Short term methane measurements is a method that utilizes spot sampling or spot measuring, which is essentially a snapshot of emissions at one moment. Short term measurements are not equal to total daily methane production as seen in respiration

chambers. These snapshots add variation that isn't seen in RC's, such as measurement times relative to feeding, amount of feed intake prior to measuring, diet selection by the animal, and level of activity prior to measuring. The main sources of variation when using an RC is relative to amount and type of diet fed. This short term, spot sampling method is done by collecting the breath of an animal at a given point, and can be done so at different times throughout the collection period (Hegarty et al., 2013).

There are different ways to collect short term measurements of methane, with one way being described by Oss et al. (2016) as a facemask (FM) collection. These authors collected emissions from bulls for one 30 minute period each day for 3 days in a row. During this period a measurement is taken every minute for 20 minutes, and ambient air was sampled for 5 minutes at the beginning and the end of the 30 minutes. It is a similar procedure to a head chamber but only done so for a short duration. This method was tested against RC and SF_6, and the results showed that all three methods were different ($P < 0.01$) from each other. Respiration calorimetry captured the greatest methane emissions, FM was intermediate, and SF_6 had the lowest measured emissions. Another short term measurement is using portable accumulation chambers (PAC) described by Goopy et al. (2011). These chambers are designed to enclose an animal for 1 or 2 hours, and take one measurement as a representative sample for the period in the PAC. The authors validated these results against 22 hour, open circuit calorimetry chamber data taken on the same sheep and expressed the relationship to determine if it will work to predict total daily methane production. They did the PAC collections for 3 days straight and found the relationship between 22 and 2 h on days 2 and 3 to be $r^2 = 0.48$ and 0.42, respectively ($P < 0.03$). Goopy et al. (2009) showed the 2 h measurement to be the best

predictor of daily methane production (DMP), accounting for 50-82% of the variation in DMP. These authors concluded that although less accurate than the open-circuit chambers, the short term measurements correlated with 22 h measurements and show potential as a predictor of DMP.

Another short term method by Garnsworthy et al. (2012) gathers emission data only at a time of feeding a concentrate supplement as they enter the milking parlor. The measurement provides an estimate of methane production rate during milking by calculating the emissions per eructation as well as number of eructations during eating. This method is not representative of DMP because it only takes place when feeding, which leads to more fermentation and therefore more eructations than other times of the day. Variation in the short term sampling is largely due to time of feeding relative to time of gas collections, making it difficult to make total daily emission assumptions or predictions. One way to limit this variation is by collecting multiple short term measurements throughout a period as a way to justify scaling up the measurements to predict daily methane emissions, which is the goal behind the GreenFeed^{TM} system (C-lock, Inc., Rapid City, SD). This system is used to estimate individual animals' DMP using short term measurements over several days. A portable automated feeding system that delivers supplement upon entering the hood is used to lure cattle into where the analyzer is located and carbon dioxide and methane flux are measured by the analyzer. Cattle enter the automated feeding system several times a day during a collection period (usually several days) and the data collected can be used to extrapolate DMP of the individual cattle (Hammond et al., 2015). The authors found no differences between treatments with the Greenfeed method, but RC and SF_6 detected a difference. The reason

for failing to pick up the difference could be due to timing and number of visits the animal makes to the hood. Another disadvantage is the variation between animals and across days, largely depending on time of feeding and time visiting the hood.

A hand-held laser technique described by Chagunda et al. (2009) represents another spot measurement method for short term methane measurements. The gun reads methane concentration in the air up to 3 meters away from the animals' nose and is calibrated for ambient levels. The readings can be done in 15 to 25 seconds. This method is quick and easy, but makes some large assumptions. To calculate total methane volume being emitted, tidal volume is needed. Tidal volume changes with changing animal behavior (standing vs. laying) and average methane emission rates are needed for time spent ruminating, feeding, standing and laying. Using these averages and assumptions, DMP is calculated by adding the emission rate of each activity and multiply it by time spent doing that activity. Although many assumptions are being made (tidal volume, animal behavior and activity), the results are highly correlated with RC emission measurements ($r^2 = 0.7$).

Short-term measurements are a common practice in methane collection, and as stated above there are many different methods for doing so. Whether it is using the CO_2:CH_4 ratio, collecting gas during feeding/milking, using the GreenFeed^{TM} system, or the laser gun method, the concepts are being adopted across many different emissions studies. Although no methodology of collection is perfect, there are many adequate methods being used to quantify methane production in ruminants.

Prediction equations for beef cattle

Predictive equations and models have been around for a long time in an effort to accurately estimate methane production from ruminants. There are both mechanistic and empirical models present as well as linear and nonlinear equations with most of the published models coming from sheep and dairy cow data. As more data have been collected using different equations, it is clear that there should be species dependent predictive equations (cattle vs. sheep), as well as dairy vs. beef cattle, which should also account for forage vs. concentrate levels in the diet. Three classical models/equations are the basis of many current methane prediction equations. Moe and Tyrelle (1979) is perhaps the most accurate of the 3 partly because it accounts for the feed quality and characteristics, such as soluble vs. insoluble carbohydrates present in the feed (hemicellulose, cellulose, cell solubles):

CH_4 (g/d^{-1}) = [3.41 + (0.511 x NSC kg/d^{-1}) + (1.74 x HC kg/d^{-1}) + (2.65 x CEL kg/d^{-1})]/0.05565

*NSC, non-structural carbohydrates; HC, hemicellulose; CEL, cellulose.

Blaxter and Clapperton (1965) developed an equation that predicts methane based off of gross energy digestibility as well as intake level relative to the animals' maintenance requirement:

CH_4 (g/d^{-1}) = [1.3 + (0.112 x ED_m, % of GEI) + MN x (2.37 – (0.05 x ED_m, % of GEI))]/[(100 x GEI MJ/d^{-1})/0.05565]

*ED_m, energy density at maintenance; MN, multiple of maintenance.

Wolin (1960) developed a model that focuses on the molar proportions of volatile fatty acids in the rumen, while assuming all excess hydrogen is found in methane:

CH_4 (mol/mol VFA) = [(0.5 x acetate) – ((0.25 x propionate) + (0.5 x butyrate))]

The Beef Cattle Nutrient Requirements Model (BCNRM) book, which summarized 16 predictive models for methane estimation into three categories: High-forage diet (≥ 40%), intermediate-forage diets (20-40%) and low-forage diets (≤ 20%). These equations are commonly used today for predicting methane emissions from beef cattle.

Equation	Reference
High-Forage Diet	
CH_4 g/d = 71.5 (± 11.5) + 0.12 (± 0.03) x BW, kg + 0.10 (± 0.01) x DMI^3, kg/d – 244.8 (± 56.44) x Fat^3, kg/d	Escobar-Bahamondes et al. (2016)
CH_4, MJ/d = DMI, kg/d x GE, MJ/kg x 0.065, % of GE	IPCC (2006, Tier II)
CH_4, MJ/d = -1.01 + 2.76 x NDF, kg/d + 0.722 x Starch, kg/d	Ellis et al. (2009)
CH_4, MJ/d = 45.98 – (45.98 x $e^{-0.003 \times MEI, MJ/d}$)	Mills et al. (2003)
CH_4, MJ/d = 2.68 – 1.14 x (Starch/NDF) + 0.786 x DMI, kg/d	Ellis et al. (2009)
CH_4 g/d = (-1.487 + 0.046 x GEI x 4.184 + 0.032 x NDF, % DMI + 0.006 x BW) x 1,000/55.65	Moraes et al. (2014)
CH_4 g/d = (-0.221 + 0.048 x GEI x 4.184 + 0.005 x BW) x 1,000/55.65	Moraes et al. (2014)
CH_4 g/d = (-0.163 + 0.051 x GEI x 4.184 + 0.038 x NDF, % DMI) x 1,000/55.65	Moraes et al. (2014)
CH_4 g/d = (0.743 + 0.054 x GEI x 4.184) x 1,000/55.65	Moraes et al. (2014)
Intermediate-Forage Diet	
CH_4, MJ/d = 2.7 + 1.16 x DMI, kg/d – 15.8 x EE, kg/d	Ellis et al. (2007)
CH_4, MJ/d = DMI, kg/d x GE, MJ/kg x 0.065, % of GEI	IPCC (2006, Tier II)
Low-Forage Diet	
CH_4 g/d = -10.1 (± 0.62) + 0.21 (± 0.001) x BW, kg + 0.36 (± 0.003) x DMI^2, kg/d – 69.2 (± 1.65) x Fat, kg/d + 13 (± 0.45) x (CP/NDF) – 4.9 (± 0.07) x (Starch/NDF)	Escobar-Bahamondes et al. (2016)
CH_4, MJ/d = -1.01 + 2.76 x NDF, kg/d + 0.722 x Starch, kg/d	Ellis et al. (2009)
CH_4, MJ/d = DMI, kg/d x GE, MJ/kg x 0.03, % of GE	IPCC (2006, Tier II)
CH_4, MJ/d = 0.357 + 0.0591 x MEI, MJ/d + 0.05 x Forage, %	Ellis et al. (2007)
CH_4, MJ/d = -1.02 + 0.681 x DMI, kg/d + 0.0481 x Forage, %	Ellis et al. (2007)

Dietary Mitigation Strategies for Methane Production by Cattle

Level of intake

Although this may not necessarily be a practical strategy, level of intake is a main determinant in the amount of methane produced by cattle. As the amount of feed intake increases, methane production also increases. Greater intake results in more fermentation in the rumen, and therefore more gas production as a by-product of fermentation. Methane production is largely dependent on the quantity of feed intake, although it can be altered by the quality and digestibility (Blaxter and Clapperton, 1965). These same authors looked at 48 trials where sheep were fed different levels of feed, and found that in all 48 cases, intake was the primary driver of methane production. Although the amount of methane produced increases with intake, the rate at which it is produced declines steadily. Johnson and Johnson (1995) found that as feeding level increases, methane lost as a % of GEI decreases by 1.6% for every percent GEI increase. The reason for this is thought to be a result of greater passage rate as feed intake increases. Level of intake has less effect on passage rate for a high forage diet compared to a high concentrate diet (Mathison et al., 1998). Boadi et al. (2004) states that 28% of methane production variation is a result of particle retention time in the rumen, and that as passage rate increases, propionate production increases, which is a hydrogen acceptor. This intake function has partly given rise to selecting cattle with lower residual feed intakes (RFI), which is described as the difference between actual feed intake and expected feed intake at maintenance given a certain body weight. This would be beneficial for reducing methane because a low RFI would mean less intake is needed to get equal performance, resulting in less methane produced. Nkrumah et al. (2006) conducted a study on methane production from low- and high-RFI cattle to examine potential differences in gas production. The authors reported a reduction in methane (% of GEI) of 25% for the low-

RFI cattle relative to the high-RFI cattle, implying that the difference is not only due to lower feed intake. The authors suggest that inherent differences in the animals could lead to differing microbial environments that has more to do with phylogenetic rather than dietary constraints. Residual feed intake is a moderately heritable trait and is seen as a possible mitigation route for methane produced by cattle (Hegarty et al., 2007).

Quality and digestibility of the diet

Concentrate-based diet

Aside from restricting intake, the most effective way to decrease emissions is to improve the quality (DEI) of the diet provided. The main way to do this is by replacing roughages with concentrates in the diet, i.e. changing the carbohydrate source from fiber to starch entering the rumen. When the fiber cell wall is fermented, the ratio of acetic:propionic acid increases in favor of acetic acid, resulting in greater methane losses. Fermentation of soluble carbohydrates produces less methane as a result of reducing acetic acid production. Additionally, as amount of carbohydrate fermentation increases, methane production decreases per unit of gross energy (GE) intake (Johnson and Johnson, 1995). Beauchemin and McGinn (2005) evaluated energy losses associated with backgrounding and finishing steers on either corn or barley based diets. The results show that the backgrounding phase had a greater methane loss per unit of GE intake (7.4%) than the finishing phase (3.4%). This can be attributed to a greater pH in the backgrounding phase vs. the finishing phase due to more roughage, leading to greater acetate:propionate levels (2.75:1 to 0.98:1).

The increase in propionate helps mitigate methane production because that process competes with methanogenesis, whereas acetate (2 carbons) promotes methanogenesis by yielding excess carbons when being converted to pyruvate (3 carbons). Methanogens typically find a pH below 6 to be toxic, which would be the case in a high concentrate diet, resulting in lower methane emissions. The finishing phase decreased methane by 38% for barley-fed cattle and 64% for corn-fed cattle over their respective backgrounding phases. There was no difference in methane emissions between corn and barley treatments in the growing phase, however, the finishing phase saw the corn diet decrease ($P < 0.05$) methane g/kg of DMI and as a % of GEI compared to the barley diet. The corn-based finishing diet saw cattle lose 2.8% of GE intake as methane which is in line with previous work stating the expected range for methane loss from cattle on diets over 80% corn is between 2 and 4% of GE (Johnson et al., 1994; Johnson and Johnson, 1995). The cattle on the barley finishing diet lost 4% of GE as methane which was lower than the expected range of 6.5 to 12% (Hashizume et al., 1968; Whitelaw et al., 1984). That range was derived from studies that do not replicate typical feedlots as Hashizume et al. (1968) used two mature cows consuming only 26-41% barley and Whitelaw et al. (1984) used cattle that were being fed at 70% of ad-libitum (Beauchemin and McGinn, 2005). Boadi et al. (2004) examined the forage:concentrate ratio effect on methane production in a feedlot setting. The high concentrate diet was 83.5% barley grain and resulted in methane production of 2.5 % of GE intake, which is lower than the 4 % of GE intake seen in Beauchemin and McGinn (2005) study and lower than the range for barley diets established by Hashizume et al. (1968) and Whitelaw et al. (1984). Lovett et al. (2003) tested three different forage to concentrate

ratios (65:35, 40:60, and 10:90) to determine the impact on methane production. As forage level decreased, methane production linearly decreased (P <0.01) for methane g/d, g/kg DMI, and as a % of GEI. Methane production would be lowered if cattle could be fed a concentrate diet that did not include a roughage source, but this would lead to acidosis. The topic of acidosis will not be discussed in detail in this review, but it is important to understand that roughages are needed in concentrate diets because they can raise the pH, even though energetically they are less efficient and produce more methane. Hales et al. (2014) tested methane production with roughage inclusion in a finishing diet when beef cattle were fed increasing levels of alfalfa displacing dry-rolled corn from 2-14%. As alfalfa levels increased, GE intake decreased (P =0.02), methane production increased (P < 0.01) linearly, and DMI was not affected. No DMI effect was observed, so the cattle were likely not acidodic.

Forage-based diets

In forage based diets, dietary NDF is the biggest driver of methane and carbon dioxide production. Johnson et al. (2002) showed that increasing forage inclusion in the diet increases greenhouse gas production in dairy cows. The authors tested three forage inclusions: 43, 61, and 96% and found that kg of carbon dioxide per kg of milk produced was 1.26, 1.38, and 1.62 respectively. These authors supplemented dairy cows with concentrate on pasture to determine the effects on methane production. A concentrate (no mention of what concentrate was used) was supplemented at 0.87 and 5.24 kg DM. Dry matter intake was greater (P < 0.01) for the high level of supplementation treatment, potentially leading to the increase (P < 0.05) in methane production (g/d) that was

observed. Typically, with greater inclusions of concentrate, methane production would be reduced but the authors stated that this concentrate source was greater in fiber than many concentrates and could help explain this response. Ominski et al. (2006) examined the effect of varying neutral detergent fiber (NDF) content in alfalfa-grass silage on growing cattle fed over a winter feeding phase. The NDF ranged from 46.4% representing the highest quality silage to 60.8% which is the lowest quality silage. The authors observed a decrease ($P < 0.01$) in DMI and ADG associated with the lowest quality silage, but did not pick up a methane production difference across NDF levels. Methane as a % of GEI decreased ($P < 0.01$) as the winter period progressed, suggesting that the cattle became more efficient as body weight increased. The methane lost as a percent of GEI ranged from 5.1 to 5.9, which is slightly lower than the 6.5% methane loss of GE intake estimated by the IPCC Tier 2 model for forage-fed cattle (Houghton et al. 1996).

Boadi et al. (2004) fed finishing cattle a high forage diet (for finishing cattle) of 42% barley silage and 42% barley grain and saw a 2% methane loss of GE intake which is lower than most forage based diets, but could be due to a greater grain component than most growing based forage diets. Boadi and Wittenberg (2002) fed low, medium and high quality forages to beef and dairy heifers and observed that the high and medium treatments increased ($P < 0.01$) methane (L/d) production over the low quality forage treatment. Waghorn et al. (2002) tested differing inclusions of fiber in 10 forage based diets consumed by sheep on methane production. The diets fed had a wide range in NDF content (13-44%) as well as CP content (12-26%). The sheep consuming *Lotus pendunculatus* (lotus) produced methane at 11.5g/kg DMI, which was the lowest of the

ten treatments while the sheep on the pasture diet produced methane at 25.7g/kg DMI. The lotus treatment contained a high inclusion of condensed tannins, which decrease methane production and had a relatively low NDF (29%). The greater NDF content on the low quality diet led to more methane produced. Promoting non-H_2-producing bacteria in the rumen can be a method for reducing methane production because H_2 production can lead to methane production. *Fibrobacter succinogenes* is one bacteria that does not produce H_2 so promoting its presence in the rumen should reduce methane production. Chaucheyras-Durand et al. (2008) conducted a lamb trial that influenced the composition of the cellulolytic flora in lambs to see the effects of fibrolytic bacteria on methane production in vitro. The two inoculants used in vitro were one favoring H_2 and one inhibiting H_2 production. The authors observed that in the reduced-H_2-inoculant lambs, methane production was lower than the H_2-favoring-inoculant lambs. This method has been tested in vitro and suggests that non-H_2-producing bacteria could be a possible method for methane reduction.

Alternative Hydrogen Sinks

Enteric methane production can be reduced in three ways: 1. Removal of methanogens present in the rumen; 2. by reducing the amount of H_2 produced in the rumen; 3. by providing H_2 with an alternate route or sink (Hulshof et al., 2015). Dietary fats have been studied as a possible strategy to reduce methane production. Johnson and Johnson (1995) describe three ways dietary fats can impact methane production: biohydrogenation of unsaturated fatty acids entering the rumen, increasing propionic acid production, and inhibiting protozoal production. Czerkawski et al. (1966) explained that

unsaturated fatty acids entering the rumen undergo biohydrogenation, which competes for hydrogen ions with methanogens, but in a small capacity. Biohydrogenation is the process of hydrogenating unsaturated fats into saturated fats in the rumen. This process is energetically favorable to the microbes, and happens to alleviate the toxicity issues that unsaturated fats have on the microbial population, primarily fibrolytic bacteria. Metabolic hydrogens used for biohydrogenation is 1% while reduction of carbon dioxide to methane is 48%. The hydrogen sink mechanism isn't thought to be the primary reason that lipid inclusion in the diet decreases total methane production in a ruminant animal. The supplementation of lipid into the diet decreases methane production and emissions by depleting methanogenic bacteria and protozoa in the microbial population, and by effectively reducing the amount of organic matter that is available for fermentation in the rumen (Beauchemin et al. 2007). Beauchemin et al. (2008a) showed that a 1% increase in dietary fat results in a 5.6% reduction in methane (g/kg of DMI). The effects on methane production can vary due to dietary inclusion of lipid supplemented, fatty acid chain length, and the interactions between lipids and the base diet (Johnson and Johnson 1995; Dong et al., 1997).

Oils/Oilseeds and FA chain lengths

One way to bring lipids into cattle diets is through oil or oilseed supplementation. This can come at the expense of DMI, diet digestibility, or both, depending on FA profile, amount fed, or composition of the diet (Beauchemin et al., 2009; NRC 2001). Van Nevel and Demeyer (1996) theorized that unsaturated fatty acids reduce methane more than saturated fatty acids. Patra et al. (2013) showed this in a meta-analysis on

saturated fats compared to mono- and poly-unsaturated fats effect on methane production. These authors observed no effect on methane production from saturated fats, but mono- and poly-unsaturated fats reduced methane (g/d; $P < 0.05$). Sauer et al. (1998) tested unsaturated fat inclusion vs. no fat inclusion by adding soybean oil to a cow diet and observed a methane reduction over the control. Beauchemin et al. (2009) examined the effect that differing fatty acid chain lengths had on methane production by feeding calcium salts (long chain fatty acid), crushed sunflower, crushed flaxseed, and crushed canola seed in a barley silage/barley grain based diet. The oilseeds added 3.1 to 4.2% fat DM to the diet. The results averaged across the oilseed treatments showed a 13% reduction ($P < 0.05$) in methane (g/d). When analyzed as a unit of intake (g/kg DMI), flaxseed reduced ($P < 0.05$) methane by 18% and canola seed by 16%. Sunflower seed numerically lowered methane by 10%. However, when methane per unit of digestible DMI was examined, only canola seed ($P < 0.05$) reduced methane while sunflower seed and flaxseed decreased digestibility by 16 and 9%, respectively, relative to the calcium salt control. Canola seeds are high in $C_{18:1\ n\text{-}9\ cis}$, flaxseeds are high in $C_{18:1\ n\text{-}7\ cis}$, and sunflower seeds are high in $C_{18:2\ n\text{-}6\ cis}$, implying that chain length in this instance did not impact methane production.

Medium-chain fatty acids (MCFA; $C_{8:0} – C_{16:0}$) were found to decrease methane production by Dohme et al. (2000), with coconut oil ($C_{12:0}$ and $C_{14:0}$) being a commonly used MCFA in cattle diets. Which MCFA is responsible for methane inhibition was examined by Dohme et al. (2001) by comparing several MCFA's against long-chain fatty acids (LCFA; $C_{16:0} – C_{18:0}$) and polyunsaturated fatty acids (PUFA; $C_{18:2}$). The study showed that C_{10} MCFA's promoted the most methanogens, while $C_{12:0}$ and $C_{18:2}$ had the

lowest methanogens in the tested rumen fluid. Palm oil ($C_{12:0}$ and $C_{18:2}$) and coconut oil reduced ($P < 0.05$) methane by 25% and 18%, respectively. The authors stated that coconut oil may suppress fiber digestion in the rumen and promote it in the hindgut, potentially leading to more methane production in the hindgut. Machmuller et al. (2000) showed that coconut oil decreased ($P < 0.01$) whole-body methane production as a unit of total energy loss, and as a % of GEI ($P = 0.04$) in lambs, which accounts for hindgut gas emissions. Dong et al. (1997) tested canola oil, cod liver oil, and coconut oil in growing and finishing diets using in vitro techniques. The growing diet consisted of 100% grass hay with an additional 10% oil sprayed on to it and the finishing diet was 90% wheat grain, 10% grass hay with an additional 10% oil sprayed on it. There was greater methane reduction in the finishing diet compared to the growing diet, but both saw significant reductions, with coconut oil being the most effective for methane mitigation. Coconut oil reduced ($P < 0.05$) methane per gram of digestible DM and total production over the control, canola oil, and cod liver oil treatments. Fiber digestion was impacted the most by coconut oil, suggesting that the reduction in methane would be at the expense of nutrient availability in a production setting. Machmuller and Kreuzer (1999) fed coconut oil to sheep at 3.5% and 7% and observed that methane production was lowered ($P < 0.01$) by 28 and 73% respectively, and cell wall digestibility was numerically reduced by 19 and 23% as coconut oil inclusion increased. Dohme et al. (2000) did an in vitro study on palm kernel oil, coconut oil, and canola oil and observed methane decreases in the order of 34, 21, and 20% respectively. In an 85% concentrate diet, Mathison et al. (1997) reported a decrease in methane production of 33% when 4% canola oil was added. However digestibility was reduced, resulting in equal

metabolizable energy intake values for the control and canola oil treatment. Engstrom et al. (1994) reported that feeding canola oil to steers tended ($P < 0.10$) to increase ADG, but did not affect DMI. The authors reported a drop in digestibility and methane output was not measured. Methanogens can be affected by fatty acids in the rumen due to a toxicity response. *Methanobacterium ruminantium* is a methanogen that is largely affected by unsaturated fatty acids, with inhibition following this order: $C_{18:1}>C_{14}>C_{12}>C_{16}>C_{18}$. Gram-positive bacteria tend to be more susceptible to the toxicity of unsaturated fats while gram-negative bacteria are less sensitive at the same concentration (Dong et al., 1997). When protected fats are fed, such as prilled, crystalline, or Ca soap fats, reduction in methanogenesis does not occur because it avoids rumen microbe interaction (Boadi et al., 2004). Hales et al. (2017) fed corn oil to steers on a finishing diet to determine if there would be an impact on methane production. The four treatments were 0, 2, 4, or 6% corn oil added to a diet displacing dry-rolled corn. Dry matter intake or GEI did not differ between treatments ($P \geq 0.39$). A linear decrease ($P = 0.01$) in methane (Mcal/d) was observed as corn oil was increased in the diet. Methane production linearly decreased ($P < 0.01$) as a function of GE intake as corn oil increased in the diet. The effect that corn oil has on methane production has not been examined thoroughly but could be a viable option for mitigation purposes in the Midwest due to the abundance of ethanol produced in the area, and thus corn oil availability. Some management /logistical issues come with feeding corn oil due to it being a liquid and potentially a DMI depressant, but nonetheless, corn oil has shown to be a viable methane mitigation option (Hales et al., 2017).

It is clear that feeding fats is a viable option if methane reduction is the main goal. However, cattle producers are unlikely to feed lipids with methane reduction being the main goal if performance or cost of gain is worse as a result. Although methane reductions of >40% are possible with lipids, 25-30% reductions are more likely for producers. Lipids should not be fed in excess of 6-7% otherwise DMI will decrease and performance can be reduced. Lipids are fed as an energy source to cattle, with the main sources coming from tallow or distillers grains.

By-products

Feeding by-products is a common practice in the beef industry, especially with the continuous rise in ethanol production over the past 15-20 years (although it has plateaued the past 5 years). Distillers grains is the main by-product of ethanol production being fed to cattle. Several distillers grains products are common, and vary depending on the moisture level: wet distillers grains plus solubles (WDGS), modified distillers grains plus solubles (MDGS), or dry distillers grains plus solubles (DDGS). In forage-based diets, distillers grains are used for energy, usually replacing some roughage or grain. McGinn et al. (2009) fed Jersey steers a growing diet consisting of 60% barley silage and either 35% steam rolled barley grain, or 35% corn DDGS. The DDGS treatment lowered daily methane emissions by 20% and as a function of DMI by 16.4% over the control. When adjusted for GE intake (about 20 MJ/kg DMI), the DDGS treatment lowered methane by 24% over the control. Foth et al. (2015) examined DDGS effect on methane production from lactating dairy cows. The diets were 24.5% corn silage, 18.4% alfalfa hay, dry rolled corn and soybean meal or 28.8% DDGS (displacing equal amounts of

DRC and SBM). The authors observed a decrease ($P < 0.01$) in methane (g/d) in the DDGS diet of 6% compared to the control. Hunerberg et al. (2013) evaluated the effect of corn or wheat-based DDGS on methane production in growing beef heifers. The control diet was 55% barley silage and 35% barley grain, and the three treatments were corn DDGS (CDDGS), wheat DDGS (WDDGS), and wheat DDGS+oil (WDDGS+oil) displacing barley grain and canola meal at 40% of the diet DM. CDDGS (21.5 g/kg DMI and 6.6% GEI) and WDDGS+oil (21.1 g/kg DMI and 6.3% GEI) reduced ($P < 0.05$) methane per unit of DMI and as a % of GEI. Wheat DDGS had no impact on methane production as a unit of DMI or GEI. All 3 DDGS treatments reduced methane on a g/d basis. Benchaar et al. (2013) fed DDGS to Holstein cows at 0, 10, 20, and 30%, displacing steam-flaked corn and soybean meal in an alfalfa silage/corn silage based diet. As DDGS inclusion increased, methane production (g/d, g/kg DMI, % of GEI, % of DEI) linearly decreased ($P \leq 0.03$). Overall, fat level of the distillers grains seems to be the reason for any decrease in methane observed. With DDG's low in fat, such as WDDGS, no drop in methane as a unit of intake or GEI was observed. Performance measures for diets containing distillers grains are also improved in most studies, which is a benefit for the producers.

In finishing diets, there has been less work examining the effect of distillers grains on methane production. Hales et al. (2012) looked at the effect of steam-flaked corn (SFC) fed with Wet distillers grains plus solubles on methane production compared to dry-rolled corn (DRC) with WDGS in finishing diets. There were 4 treatments: 1. SFC with 0% WDGS; 2. SFC with 30% WDGS; 3. DRC with 0% WDGS; and 4. DRC with 30% WDGS. The diets were balanced for RDP by adding cottonseed meal, and yellow

grease was added to balance fat (roughly 6% fat in each diet). Methane production (L/steer, L/kg DMI, Mcal, % of GE, % of DE intake) was reduced ($P \leq 0.05$) for SFC diets than DRC diets, and energy retention was greater for SFC diets illustrating that the more nutrient-available grain led to better energy utilization. WDGS had no effect ($P \geq 0.55$) on methane production regardless of how it was presented (L/steer, L/kg DMI, Mcal, % of GE, % of DE intake). Hales et al. (2013) examined the effect of WDGS included at different inclusion in SFC-based diets on methane production in finishing steers. The 4 treatments were 0, 15, 30, or 45% WDGS displacing mostly SFC in the diets. The control was balanced for RDP by adding cottonseed meal at 5.60%. Fat was attempted to be balanced across treatments by adding yellow grease to the control, but the 30 and 45% WDGS treatments exceeded recommended values with a fat content of 7.4 and 8.3% respectively (control was 5.9%). Methane production increased linearly ($P < 0.01$) as a function of DMI, GE, or DE as WDGS inclusion increased. Carbon dioxide decreased linearly ($P = 0.05$) as inclusion of WDGS increased. Retained energy as a percent of GE decreased linearly ($P = 0.04$) as WDGS inclusion increased, likely due to fecal energy lost that is associated with feeding WDGS. As WDGS increased, NEg of the diet decreased from 2.02 at 15% WDGS to 1.38 at 45% WDGS, meaning that SFC-based diets with WDGS should be adjusted for NE_g depending on the inclusion of this by-product. The concept of decreasing methane with distillers grains in a finishing diet is intriguing because it replaces starch with fat, but also with fiber and could theoretically balance one another out and have no impact on methane production. The results vary when examining methane production with distillers grains in the diet across growing and finishing work and could use further examination in the future.

Hales et al. (2015) examined the effects of corn processing method and inclusion of WDGS on methane production using 6 hr headbox collection periods to measure emissions. The treatments were DRC with 25 or 45% WDGS, or HMC with 25 or 45% WDGS. Methane production as a % of GEI was reduced ($P < 0.02$) for the DRC and HMC + 45% WDGS diets relative to the diets containing 25% WDGS. When methane loss (Mcal) was analyzed, DRC + 45% WDGS and HMC + 25% WDGS treatments were lower than the other two treatments ($P < 0.01$), although HMC + 45% WDGS was numerically reduced by 10%.

Nitrates

Nitrates have become another viable option for methane mitigation by acting as an alternative H+ sink. In order for an alternative electron acceptor to be capable of competing with methanogenesis, it must be more energetically favorable than production of methane from carbon dioxide (Hulshof et al., 2012). When nitrates are converted to ammonia, 4 moles of hydrogen are used in nitrate reduction rather than carbon dioxide reduction, therefore methane production should be lowered by 1 mole for every mole of nitrate reduced. This is equivalent to reducing methane emissions by 25.8 g for each 100 g of nitrate fed in theory (Van Zijderfeld et al., 2010). Takahashi and Young (1991) first reported methane reduction from nitrates in sheep, and further studies have followed but not until recently in beef cattle. One concern with dietary nitrates is methemoglobinemia accumulation in the blood, but can be avoided by adapting the animal to nitrates over a period of time (Hulshof et al., 2012).

Van Zijderfeld et al. (2010) tested the effect of nitrate in the diet (2.6 % of DMI) on methane production in lambs. A reduction in methane of 32% ($P < 0.01$) was observed when analyzed as liters/day. Methane was reduced ($P < 0.01$) as a function of DMI as well as metabolic body weight and DMI was not impacted by including nitrates in the diet. Hulshof et al. (2015) tested the effect of dietary nitrate supplementation on methane production in beef cattle on sugarcane/corn/soybean-based diets. Nitrates were included at 22g/kg of DM and cattle were allotted a 16 d adaptation period. Methane (g/d) was reduced ($P < 0.01$) by 32% when nitrates were fed compared to the control diet. Methane was reduced ($P < 0.01$) in the nitrate diet by 27% as a function of DMI and from 5.9 to 4.2 as a % of GEI. Dry matter intake tended ($P = 0.09$) to decrease when nitrates were present in the diet. Methane was reduced to 87% of the theoretical potential that was previously discussed. Van Zijderfeld et al. (2011) fed nitrates to dairy cows at 21g/kg DM to determine the mitigation effect on methane. Nitrates decreased ($P < 0.01$) methane production by 16% when looked at in g/d, g/kg DMI, or as a percent of GEI. Dry matter intake was not impacted by feeding nitrates. Guyader et al. (2015) examined the effects of feeding linseed oil and calcium nitrate, either in combination or separate, to non-lactating cows on methane production. The oil was fed at 4% DM and nitrate was 3% of diet DM in a 50:50 grass hay:concentrate based diet. The linseed oil and calcium nitrate treatments when fed separate decreased ($P < 0.01$) methane (g/kg DMI) by 17 and 22% respectively. When fed in combination, methane (g/kg DMI) was reduced by 32%. Linseed oil and calcium nitrate both decreased ($P < 0.01$) methane as a % of GEI when fed separate but in combination did not have an effect ($P = 0.24$). Dry matter intake was not impacted by either of the additives. Troy et al. (2015) fed nitrates and rapeseed cake

(source of oil) to cattle fed either a 50:50 roughage to concentrate (growing) diet or a 8:92 roughage to concentrate (finishing) diet to determine if methane production would be affected. Calcium nitrate was fed at 2.15% of diet DM and rapeseed cake increased dietary oil to 5.3% of diet DM. The steers fed the finishing diet produced less ($P < 0.01$) methane and H_2 than the growing diet when analyzed as g/d, g/kg DMI, and as a % of GEI. The growing diet showed a decrease ($P < 0.01$) in methane (g/kg DMI) of 17% when nitrate was added while rapeseed cake did not affect methane production statistically ($P = 0.18$), but did lower it by 7.5% numerically. In the finishing diet, neither nitrate nor rapeseed cake lowered methane production. With limited work done on finishing cattle given nitrate, it is hard to tell why methane was not reduced. In growing diets the data suggest that nitrate supplementation lowers methane but this may not be the case for finishing diets. Total methane produced in finishing diets (on a % of GEI and sometimes a g/d basis) is lower than growing diets, suggesting that because there is less methane present it may be difficult to decrease even more with nitrate supplementation. More work is needed on this topic, but it shows promise in the growing cattle sector as a viable mitigation option.

Sulfates

Sulfates have been used as an alternative H+ sink for similar reasons as why nitrates are used. Sulfate reduction to H_2S uses 8 electrons in this process, which is equal on a per mole basis to nitrate being reduced to ammonia. Sulfate reduction to hydrogen sulfide is more energetically favorable than methanogenesis and theoretically should decrease methane by 16.7 g per 100 g of sulfate reduced (Van Zijderfeld et al., 2010).

One concern with feeding sulfate is the risk of polioencephalomalacia (polio) which can be caused by H_2S, so an adaptation period is recommended (Sarturi et al., 2013). Sulfate reducers are generally able to outcompete methanogens because they are capable of using hydrogens at lower partial pressures. Once sulfate is reduced to sulfite it can become toxic to methanogenic bacteria, offering another form of methane mitigation aside from being an electron acceptor. Due to sulfur toxicity levels being only 0.4% of the diet DM, there is limited potential to use it as a strategy for methane mitigation (Mathison et al., 1998).

Van Zijderfeld et al. (2010) fed sulfate to sheep at 2.6% of the DM and were adapted to the diet over a 4 week period. Sulfate decreased ($P < 0.01$) methane production (L/d) by 16% over the control diet. Methane was reduced ($P < 0.01$) as a function of DMI as well as L/kg of metabolic BW over the control while DMI was not impacted by sulfate supplementation. This study found that methane production was reduced by 67% of the theoretical potential. The authors recognized that sulfur was fed well above recommended levels, but polio was not an issue because the diet was high in NDF, but if fed in a typical diet polio could have been an issue. This same study also fed nitrate at 2.6% of diet DM in combination with sulfate and saw a decrease in methane production of 47%. A similar study was done by Pesta et al. (2015) examining the effect of nitrate and sulfate supplementation, individually and in combination, on finishing steers. Sulfate was included in the diet at 0.54% DM and nitrate was included at 2% diet DM. No methane reduction was observed due to sulfate or sulfate + nitrate supplementation. The methane collection method was short-term measurement of methane using a calan-gate system with rubber mats to enclose the headspace. The

authors concluded that the method is not robust enough to detect differences, as other literature would suggest a difference should have been detected. There is a limited body of work on the effects of sulfate supplementation on beef cattle for methane mitigation purposes. The work that is available shows less promise than other strategies for reducing methane production in beef cattle.

Rumen Modifiers/Methanogenesis Inhibitors

As discussed at the beginning of this review, methanogens are the reason that methanogenesis takes place in the rumen. If something were to inhibit methanogens it would be perhaps the most direct way to reduce methane production. There are products called rumen modifiers that alter the microbial population in a way that can lead to less methane production. Perhaps the most widely used rumen modifier is the ionophore monensin, which is used to shift the microbial population as well as promote more propionate production. A feed efficiency response is typically observed when feeding monensin due to a drop in DMI but no change or an improvement in ADG. Apart from the performance boost, a reduction in methane production is commonly observed with monensin in the diet, although it can be a short-lived response. Johnson and Johnson (1995) claimed that monensin reduced methane up to 25%, but has been shown to only sustain this inhibition for 2 weeks due to the ability of the microbes to adapt to the presence of an ionophore.

Ionophore

Odongo et al. (2007) examined the effects of long-term monensin supplementation to dairy cows on methane production. The cows were fed a diet

consisting of 60% roughage (corn silage, haylage) and 40% concentrate (HMC) and saw a 7% reduction in methane (g/d) due to monensin inclusion. This reduction was sustained for 6 months which is in agreement with some previous work (Rogers et al., 1997), meaning that adaptation to the ionophore could be diet dependent or that a high roughage diet prolongs the effects of monensin on methane production. Guan et al. (2006) examined the short and long-term effects of feeding monensin to Angus steers. Cattle were randomly given either a low or high concentrate diet and received either monensin or lasalosid ionophores. The ionophores decreased ($P < 0.05$) methane (L/kg DMI or as a % of GEI) by 27% in the first two weeks for the high-concentrate diet, and 30% in the first 4 weeks for the low-concentrate diet. Original methane production levels returned after 3 and 6 weeks for the low-concentrate and high-concentrate diets, respectively. The authors theorized that adaptation to the ionphores by the microbes may be related to diet composition. McGinn et al. (2004) reported that monensin tended ($P = 0.09$) to decrease methane as a % of GEI by 9% and tended ($P = 0.08$) to decrease methane per kg of DMI by 8.6% in beef cattle. Appuhamy et al. (2013) conducted a meta-analysis on the effect of monensin on methane production in both dairy and beef cattle. The beef cattle that consumed monensin had methane production reduced ($P < 0.01$) by 19 g/d. Overall, monensin's effect on methane production varies across cattle type and diet composition. More work is needed to conclude an impact when monensin is fed. Feeding monensin is a strategy that shows some promise for methane mitigation, and is already widely used by producers due to the efficiency response.

BES

Another rumen modifier/methanogen inhibitor is a product called alpha-bromoethanesulfonic acid (BES) and works as an inhibitor to methanogenesis. It inhibits the growth of *M. ruminantium* and other methanogens because it is the structural analog to HS-coenzyme M, which is used by methanogens. This product showed a lot of promise as it was cheap, water soluble, and abundant, but has not been used commonly because microbes were able to develop a resistance to BES (Mathison et al., 1998). Further work showed that the proportion of methane in the rumen dropped from 40% to 1% after dosing BES at 2 g/d, but microbes were able to adapt within 4 days (Immig et al., 1996). Another compound that has been investigated as an antimethanogenic substance is bromochloromethane (BCM). McCrabb et al. (1997) did an in vivo study using BCM complexed with alpha-cyclodextrin due to BCM toxicity issues and saw a decrease ($P < 0.01$) in methane (mL/min) over 28 days on a low quality roughage diet. Hristov et al. (2013) reported that BCM reduced methane up to 50% in vivo. Although there is a concern that the microbes adapt to this class of compound, BCM has been shown to persist in the rumen. There are environmental concerns with this product as it has negative effects on the ozone, and it is currently banned which makes it difficult to pursue as a mitigation strategy.

3NOP

3-nitrooxypropanol (3NOP) has been investigated as a methane inhibitor thoroughly in recent years. Hristov et al. (2015) fed 3NOP to dairy cows at 4 different rates (0, 40, 60, and 80 mg/kg of diet DM) for 12 weeks and observed a 30% methane reduction while not impacting feed intake. Duin et al. (2016) showed that 3NOP

specifically focuses on methyl-coenzyme M reductase by binding to its receptors, which is in close proximity to nickel, allowing the nitrate group to be reduced. Lopes et al. (2016) fed 3NOP to dairy cows at 60 mg/kg of dietary DM and observed a reduction ($P < 0.01$) in methane (g/d) of 31% compared to the control. Methane production was also reduced ($P < 0.01$) when analyzed as a function of intake (g/kg of DMI) and DMI was not different. Haisan et al. (2014) conducted a study to examine the effect of feeding 3NOP on methane production to dairy cows. 3-nitrooxypropanol was supplemented at 2,500 mg/d and methane measurements were taken on d 23 and 27 using the SF_6 collection method. Methane production (g/kg DMI) was reduced ($P < 0.01$) from 17.8 to 17.18 when fed 3NOP while not impacting DMI and the acetate:propionate ratio decreased ($P = 0.04$). Reynolds et al. (2014) gave dairy cows either 0, 500, or 2500 mg/d of 3NOP in a maize silage-based diet and reported decreases in daily methane production of 6.6 and 9.8% for treatment 500 and 2500 respectively, which is considerably lower than the results from Haisan et al. (2014).

3-nitrooxypropanol's effect on beef cattle was evaluated by Romero-Perez et al. (2014) when Angus heifers were fed a diet consisting of 60% barley silage and 35% barley grain. The cattle were supplemented with four levels of 3NOP: 0, 413, 1238, and 2475 mg/d. Methane (g/d, g/kg DMI, and as a % of GEI) decreased linearly ($P < 0.01$) as inclusion increased, while DMI was not affected. Jayanegara et al. (2017) conducted a meta-analysis on 3NOP effects on methane production from dairy cows, beef cattle, and sheep. 3-nitrooxypropanol inclusion ranged from 0 to 280 mg/kg DMI. The authors reported that CH_4/BW, CH_4/DMI, CH_4/milk produced, and CH_4/GEI were all reduced ($P < 0.05$) with increasing inclusion of 3NOP in the diet.

Biochar

A product called biochar has recently become of interest due to its potential as a rumen modifier aiding in methane reduction. Hansen et al. (2012) defines biochar as a carbonized plant material produced by pyrolysis of cellulose-rich biomass. The product is porous and can either be charcoal or activated carbon, depending on the temperature used to make it. The initial substance used to make biochar can vary from wood to bone to rice hulls being just a few options. The mode of action is not completely understood, but there are several theories as to how methane can be decreased using biochar. Biochar is commonly used in soil to improve the soil properties, adsorb gas, and store carbon, and has been theorized that the gas adsorption could happen in the rumen and decrease methane eructation. Leng et al. (2012) has multiple theories as to how biochar could reduce methane. One theory is that adding biochar will increase the amount of inert surface area in the rumen where microbes can reside. With these surface areas in close proximity to the digesta suspended in the fluid, biochar will adsorb the hydrogen produced from soluble feed particle fermentation. Another theory is that the porous nature of biochar provides the microbes with habitat for feeds to be digested more completely. It is also suggested that methane oxidation may take place in this habitat by bringing methanogens and methanotrophs together, leading to more oxidation due to a greater presence of methanotrophs from biochar than in non-biochar rumens. Microbes are more reduced than their substrate, so increasing methanotrophs could also be working as a hydrogen sink. It has been hypothesized that methanotrophs form on inert surfaces when methane is present, leading to more methane oxidation and overall microbial

growth efficiency. Microbial growth efficiency could be increased with biochar by providing a closer association of the microbial colonies (Leng et al., 2012).

Hansen et al. (2012) conducted a study to evaluate the effects of biochar on methane production in vitro. Rumen fluid was collected from two Jersey steers consuming a grass silage supplemented during daytime grazing. Three types of biochar were examined with the substrate used for biochar production being wood, straw, gas, as well as a 4th treatment of activated charcoal. The diet used was total mixed ration with biochar included at 9% of diet DM. The feed was placed in a bag and put in the in vitro bottle with inoculum, and biochar was added to the bottle outside of the bag. The biochar addition to the diet did not statistically decrease methane production but did numerically reduce methane production between 11 and 17%. Activated carbon tended ($P = 0.09$) to decrease methane production per gram of feed DM and per gram of degradable DM by 20 and 33%, respectively. Leng et al. (2012) conducted an in vitro study evaluating the effect of biochar on methane production using inoculum previously adapted to biochar vs inoculum not adapted to biochar. The diet was 70% cassava root meal, 26.5-28% cassava leaf, and 2% urea while biochar was added at 1.5% of the diet DM. The rumen fluid inoculum came from steers either consuming 0.62% biochar or no biochar. The rumen fluid from biochar-adapted cattle reduced methane ($P < 0.01$) as a percent of total gas over rumen fluid without biochar. Biochar in the in vitro bottles reduced methane ($P < 0.01$) as a percent of total gas produced over the treatment without biochar inclusion. The combination of biochar in the rumen fluid and in the in vitro bottle reduced methane by about 12% while biochar added in vitro to rumen fluid absent of biochar reduced methane by 6%. Biochar increased digestibility ($P < 0.01$) when it was in the rumen

fluid, and tended ($P = 0.09$) to increase digestibility when added to the in vitro bottles.

Leng et al. (2012) fed biochar to cattle to evaluate the effects on methane production and performance. The diet fed was cassava root chips ad libitum and fresh cassava foliage at 1% of live weight (DM basis). Biochar was added to the diet at 0.6% of the diet and was made from rice hulls and another treatment was adding potassium nitrate at 0.6% of diet DM. Live weight gain increased ($P = 0.06$), feed conversion improved ($P = 0.03$) and DMI was not impacted with biochar inclusion. Methane, measured using a gas chromatograph, was decreased (ppm; $P = 0.07$) when biochar was added while carbon dioxide production increased ($P < 0.01$), leading to a lower ($P < 0.01$) CO_2:CH_4 ratio for the biochar treatment. Biochar plus nitrate showed an additive response by reducing methane by 41% compared to the control.

Plant Compounds

Using plant secondary compounds, such as tannins or saponins, as a methane mitigation strategy is relatively new and has shown some potential. Saponins have a negative effect on protozoa (alter cell wall permeability) as well as limit hydrogen availability as more propionate is produced (less protozoa = less methanogenesis) in the rumen and can therefore lead to methane reduction in some instances (Beauchemin et al., 2008: Guo et al., 2008). Holtshausen et al. (2009) fed two sources of saponin products to dairy cows and in vitro to examine their effects on methane production and fiber digestion. Saponins were included at 15, 30, and 45 g/kg substrate (DM basis) and were added to the rumen fluid inoculum in the bottles. These authors observed a linear decrease ($P < 0.01$) in methane (mg/g DM) as saponin inclusion increased. The cows

were fed saponin at 10 g/kg (DM basis), which is lower than the in vitro study to avoid potential negative effects on nutrient digestion. Methane production (g/d, g/kg DMI) did not differ when saponins were included in the diet. The authors concluded that the methane reduction observed in vitro is a function of decreased fiber digestion, whereas in vivo, the inclusion was low enough to avoid a decrease in digestion, resulting in no methane reduction. Hook et al. (2010) explains that if methane can be reduced by saponin inclusion at the expense of nutrient digestion, then it is not practical for producer adoption of this mitigation strategy.

Compressed tannins (CT) are another secondary plant compound with potential for methane reduction due to decreased availability of H_2 as well as methanogen inhibition (Hook et al., 2010). Pachula et al. (2005) fed a CT-containing forage to goats to observe any effect on methane emissions. The CT treatment decreased ($P < 0.01$) daily methane production, as a function of DMI, and digestible DMI by 43, 57, and 50%, respectively. A greater ($P = 0.01$) DMI was observed for the CT treatment over the control, but the authors state that this has been observed in previous work when feeding plants high in CT. Woodward et al. (2001) reported a decrease in methane production of 24-29% relative to digestible DMI in sheep fed CT, and 23% relative to DMI in cows fed CT. Beauchemin et al. (2007a) fed CT from quebracho trees at 0, 1 and 2% of the diet DM to beef cattle to determine if methane production would be affected. Methane production, DMI, and DMD were not impacted by CT inclusion. Crude protein digestibility decreased linearly ($P < 0.01$) as CT inclusion increased. Ebert et al. (2017) evaluated the effects of CT's on methane production in finishing steers fed a steam-flaked corn diet. The CT was included at 0, 0.5, and 1% of the diet DM. Methane

production, DMI, ADG, and feed efficiency were not impacted by inclusion of CT. The mode of action on methane mitigation from CT's in unknown, but a theory is that they have a negative (potentially toxic) effect on methanogens, which would lead to less H_2 in the rumen, resulting in less methane. More work is needed to fully understand this process because CT's do show some potential as a mitigation strategy.

Essential oils are a natural secondary plant compound and can be used as a feed additive for ruminants. Essential oils protect the plant from bacteria, fungi and insects, meaning they have antimicrobial properties. The antimicrobial capabilities of essential oils have been shown in literature to inhibit yeasts and bacteria as well as control gas production. (Boadi et al., 2004). Essential oils generally inhibit gram-positive bacteria, similar to ionophores, but some of the smaller essential oils can interact with gram-negative bacteria (Cobellis et al., 2016). Lee and Ha (2002) conducted an in vitro study with essential oil inclusions of 0, 1, and 10% in a 0.5 g sample. Methane production was reduced over 48 hours with the 10% supplement compared to 0, but 1% treatment had no effect compared to 0. Broudiscou et al. (2000) tested 13 different plant extracts to determine if they would have an impact on methane production in vitro. Methanogenesis was decreased by 14.2% by *Equisitum arvense,* and increased by 13.7% from *L. officinalis*, showing that the mode of action may be different between different plant compounds. Cobellis et al. (2016) conducted an in vitro study testing the impact on methane production of 7 different essential oils (oregano, rosemary, Ceylon cinnamon, cinnamon leaves, cinnamon bark, dill seeds, and eucalyptus). They were tested individually at inclusions of 1.125 ml/L of culture, as well as in three-way combinations at 0.8 ml/L of culture. All individual and combination essential oil treatments decreased

($P < 0.01$) methane production and total gas production (mL). However, all treatments (apart from the Ceylon cinnamon-dill seeds-eucalyptus combination) also decreased ($P < 0.01$) DM digestibility. The microbial population was altered by essential oil inclusion as archaea, protozoa and some bacteria species were reduced ($P < 0.01$). The authors concluded that the Ceylon cinnamon-dill seeds-eucalyptus combination at low levels may be a viable option for methane mitigation but more research is needed on this topic as it is not fully understood.

Acetogenesis, Propionate enhancer, Defaunation

Acetogenesis is a methane mitigation strategy that in theory shows some promise. The process diverts electrons from the methanogens by promoting acetic acid production from acetogens. Acetogens convert H_2 into acetate which can then be used by the host animal, and has been shown in termites and rodents but is also being explored in ruminants. If reductive acetogenesis can be established in the rumen, more acetate would be produced and could increase animal performance as well as decrease methane emissions (Joblin, 1999). However, in ruminants, acetogens are unable to effectively compete with methanogens, due to the low affinity of acetogenesis for H_2 (Boadi et al., 2004). Joblin (1999) states that acetogen population in the rumen is highly dependant on the diet fed. A concentrate diet has been shown to have acetogen populations > 10^8/g digesta, whereas in a forage-based diet the density of acetogens was < 10^2/ g digesta. Boadi et al. (2004) states that increasing the density in the rumen in possible through exogenous inoculation, although previous attempts at this process have been unsuccessful. Nollet et al. (1998) placed acetogen inoculant into the rumens of two rams

and observed a decrease in methanogenesis of 80%, however after 6 days methanogenesis levels of the treatment were equal to the control, implying the effects on methane inhibition are transient. The inoculant was placed in the rumen through the fistula on d 1 of a 10 d trial and was not continually added. The authors state that the loss of inhibitory effect of the inoculum after day 3 could be due to bacterial adaptation to the inoculum, or due to the inoculum being metabolized by the microbes.

Adding propionate enhancers to the diet is a strategy for methane reduction because propionate is an alternative hydrogen acceptor. Feeding an organic acid, such as fumeric acid, can lead to more propionate production because fumeric acid is an intermediary in the propionic acid pathway (Boadi et al., 2004). Wood et al. (2009) supplemented both free and encapsulated fumeric acid to growing sheep at a rate of 100g/kg and found a 62 and 76% reduction in methane production respectively for the two fumeric acid treatments. McGinn et al. (2004) supplemented growing cattle with fumeric acid at 80 g/d and saw no reduction in methane g/d, g/kg DMI, or as a % of GEI. Beauchemin and McGinn (2006) supplemented growing beef cattle with 175 g/d of FA and observed no reduction in methane production. Foley et al. (2009a) did a study where malic acid was supplemented to dairy cows on pasture at 480 g/d and no decrease in methane production was observed. Foley et al. (2009b) examined the effects of malic acid on beef cattle methane production. Malic acid was supplemented at 0, 3.5 and 7.5% of diet DM in the first experiment and 0, 2.5 and 5% in the second experiment. Experiment 1 saw a reduction ($P < 0.01$) of methane g/d and g/kg DMI of 16 and 9% respectively. Dry matter intake was also reduced ($P < 0.01$) as inclusion of malic acid

increased. The second experiment also decreased daily methane production; however DMI decreased, suggesting the methane reduction was from lower intake.

Defaunation is the removal of protozoa from the rumen by using dietary or chemical agents and has been shown to reduce methane production. Protozoa are closely related with methanogenesis as they provide a habitat for 20% of rumen methanogens and can account for 37% of methane production, so removing protozoa should decrease methane production (Boadi et al., 2004). Hegarty et al. (1999) states that in the absence of protozoa, methane is reduced by 13%, but is variable depending on the diet. The decrease in emissions as a result of defaunation can be from four mechanisms: 1) Decrease in DMD, 2) Decrease in methanogens present in the rumen, 3) A shift in VFA production and therefore hydrogen availability, 4) Increasing ruminal partial pressure. The same authors state that a high-concentrate diet results in less methanogenesis because many starch-fermenting bacteria do not produce any H_2. Boadi et al. (2004) state that a complete eradication of protozoa is not recommended because fiber digestion may be reduced. However, protozoa have negative effects on protein metabolism, resulting in less microbial protein entering the small intestine, so there are multiple considerations necessary if this practice were to be applied. Overall, it is a strategy that shows some promise for methane reduction, but more work is needed to test the applicability of it.

Literature Review Summary

Methane mitigation is a topic that is garnering a lot of attention worldwide due to the environmental concerns associated with increasing levels of natural and anthropogenic methane. Ruminant livestock animals contribute to the global methane

budget, and therefore are a central focus of the mitigation efforts. Cattle, in particular, emit a large amount of methane relative to other ruminants, which is not only an environmental concern but also an energetic loss to the animal, compromising efficient production. One of the best ways to reduce an animal's carbon footprint is by making it as efficient as possible, and that is where nutrition can make an impact. Many studies have been done in the past analyzing certain ingredients effects on methane production on a small-scale level. There is a need for practical mitigation strategies that could be adopted by the industry without compromising the production of the animal. In order to come up with these applicable strategies, more robust methods for collecting methane that simulate current production practices are needed. For feedlot cattle, measuring methane on a pen-scale would be beneficial because cattle are in a familiar environment rather than being isolated, which is common for calorimetry collections. Verification of the pen-scale calorimetry chamber against proven collection methods, such as indirect calorimetry chambers (gold standard) is important for validation of the results. Thus, one objective of this thesis is to explain the construction of a collection chamber that is applicable to industry practices. Another objective is to explore methane mitigation strategies in a newly constructed pen-scale indirect calorimetry chamber using dietary interventions in growing and finishing cattle. The final objective is to test biochar's effects on methane production using respiration calorimeters.

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CHAPTER II. Construction of a Pen-Scale Methane Measuring System

T.M. Winders, S.C. Fernando, G.E. Erickson, R.R. Stowell

Department of Animal Science, University of Nebraska, Lincoln, NE 68583-0908

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[2]Corresponding author: C220 Animal Science Building, Phone: 402-472-0726; Fax: 402-472-6362; email: richard.stowell@unl.edu

Abstract

Methane production is commonly associated with beef cattle production due to the negative environmental effects due to methane having a global warming potential 25x greater than carbon dioxide (Trotsenko and Murrell 2008). For that reason, large efforts have been made to mitigate methane from livestock systems through numerous ways. In order to mitigate methane emission it must be accurately measured in the first place. Methane emission from cattle can be measured using a number of different methods. The 'gold standard' method is respiration calorimetry, allowing for frequent measurements to occur (Pickering et al., 2013). These systems can be open- or closed-circuit, with open-circuit being more common today (Judy et al., 2017). Headboxes are commonly used respiration calorimeters, but have limitations as they do not represent cattle in a typical production setting (Madsen et al., 2010). The methane barn was developed to remedy the shortcomings of other methods, and to be more applicable to industry practices. Two side-by-side rooms sharing a central wall were converted into large open-circuit indirect calorimeters. Gravity wall inlets allow for air to enter the fully enclosed chambers via negative pressure created by two exhaust fans pulling air through each room. 'Outlet air' from each chamber is continuously extracted from an indoor location near the exhaust fans – after adequate mixing within the room – while 'inlet air' is drawn from a central outdoor location on the opposite side from the fans. Sample airstreams from each chamber and the outdoors are pumped to a common station that cycles air to a separate room with gas analyzers. An LI-7700 Open-Path CH_4 Analyzer receives air on a rotation between the two chambers and the ambient line, with ambient air being used to flush the

system between chamber measurements. Gas emissions are then calculated based upon the measured ventilation rate [held constant], gas concentration, and air properties. Dietary mitigation effects can be tested on multiple animals at the same time in this facility with confidence that differences can be detected due to the precision of the analyzer and system capabilities.

Key words: calorimetry, cattle, methane

Introduction

Methane (CH_4) production by cattle is as an energetic loss to the animal as well as a concern for the environment because of its warming potential as a potent greenhouse gas. Ruminants have been estimated to contribute around 16% of total methane production on a global scale, and are considered one of the main sources of anthropogenic methane production (Eugene et al., 2008; Mathison et al., 1998, Moss et al., 1992). Methane production is not only an environmental loss, but also an energetic loss to the animal. In theory, more methane loss could result in less energy retention and, therefore, lower productivity of the animal. Regardless of the reasoning behind the need for methane mitigation, an accurate gas collection method is essential in order to correctly measure and quantify methane production.

The 'gold-standard' method for measuring methane production from cattle is using respiration calorimetry, in which the animal is typically subject to 1-3 day collection periods with the capabilities of frequent gas sampling. Numerous different styles of respiration calorimeters are used to measure emissions from livestock. Some are indirect calorimeters that enclose just the neck and head of the animal; these are often referred to as headboxes and are described by Foth et al. (2015) and Judy et al. (2017). Respiration calorimeters can be categorized as either open-circuit or closed-circuit systems. Open-circuit calorimetry is more commonly used today and is when air is continuously circulated through the apparatus. Open-circuit systems must take into account the flow rates of incoming and exiting air and analyze the airstream prior to entering and as it leaves the apparatus. There are different types of open-circuit

calorimeters based upon how the animal is confined during collections. A closed-circuit system is when the air that enters the apparatus is recycled back to the animal after it has been analyzed and uses absorbents to remove water vapor and carbon dioxide from the air (Judy, 2017). There are open-circuit respiration calorimeters that operate on an individual-animal basis, but enclose the animal within whole-body chambers (Derno et al., 2009; Blaxter, 1962).

Respiration calorimeters do have some limitations related to animal stressors from this method. Respiration chambers are typically made for a single animal at a time. Taking an animal from a pen and isolating it during the collection period puts the animal in an uncomfortable environment. This can be combatted by acclimating the animals to the respiration chambers prior to the collection period, but this does not ensure normal behavior from the animal. According to Beauchemin and McGinn (2005), a drop in intake occurred during both growing and finishing aspects of a trial evaluating the effects of corn- and barley-based diets on methane production. Dry-matter intake was reduced by 31 and 22% during the growing and finishing phases, respectively, and the authors attributed this to the change in animal environment. Measuring animals on an individual basis is not the most applicable to how cattle are raised in the industry. Another limitation in this method is relative to animal movement, as energy expenditure would be greater in a production-type scenario where the animals would walk to water and feed, but in an open-circuit respiration calorimeter no walking occurs, which likely alters energy expenditures. Another limitation, as previously mentioned, is the inability to account for hind-gut fermentation in a headbox style open-circuit collection chamber

(Tremblay and Masse, 2016). Headboxes have been reported to have greater measurement accuracy than tracer gas methods because controlling ventilation rates is more accurate (Pinares-Patino et al., 2011). The need for production-scale methane measurement is also important to relate study results back to industry practices. Consideration of these limitations led to the development of the methane barn. The objective of this paper is to illustrate how the methane barn was constructed as well as to address what purposes it will serve for methane research going forward.

Methods

Construction and Pen Design

Two open-circuit indirect calorimeters were built by retrofitting a barn with two enclosed pens into the methane barn at the Eastern Nebraska Research and Extension Center, near Mead, NE (Figure 1). Each pen is 15.2 m long (east to west) x 13.3 m wide (north to south) with a 4.4 m wide alley running east to west on the north end of the pen. The two chambers share one central wall and a sliding alley door. Each pen has another wall and alley door on the opposite side and two doors in the south wall. The dividing walls between pens are hollow, wood-frame construction with a fabric liner and wood plank covering each side, which was beneficial for restricting air exchange between chambers. Each alley door is 3.7 m wide and 2.7 m tall. The alley doors between the pens were lined with new weather stripping (Grainger Industrial Supply, Lake Forest, IL) to stop airflow between pens, while all other observable gaps in each pen enclosure were filled with insulating spray foam sealant (Dow Chemical Company, Midland, MI). The doors in the south end of each pen are all garage doors (with 3.7 m x 2.7 m maximum

openings) that can be remotely opened and closed to facilitate delivery of feed into bunks placed just inside the doorways. The doors are normally in the closed position during trials and seal with rubber tubing along the base of the door. Air leakage around these doors was considered a negligible concern, since the objective was to have fresh air enter through openings in the south wall. Above each garage door is a gravity air inlet (2 per pen; TJW Wall Inlets, QC Supply, Schuyler, NE) that is 111 cm long x 32 cm tall.

Each chamber is equipped with two fans in the north wall, creating a negative-pressure ventilation system in which air is exhausted from the chambers through the fans and fresh, outdoor air is pulled into the chambers primarily through the gravity air inlets. Each chamber has a 25-cm fan and a 30-cm fan (Multifan System 1, QC Supply) installed side-by-side in the wall with the ability to operate them singly or together. The in-situ fan output of each fan was measured on two occasions, once on April 11, 2016, and again on August 17, 2017. The output of each of the fans was determined by Iowa State University engineers using a FANS system, which is a recognized approach for measuring fan airflow rates in on-farm ventilation and emissions research. The results of the first and second tests were similar, averaging 1274 L/s with both fans running per pen. Two ceiling fans (Grainger Industrial Supply) with a blade diameter of 305 cm were installed over each pen to ensure that air is mixed thoroughly prior to being sampled for methane and carbon dioxide.

The two feed bunks within each pen are each 3.7 m long. One water trough with a length of 137 cm, width of 84 cm, and capacity of 61 L (Ritchie Industries Inc., Conrad, IA) is located between the bunks within each pen. A concrete feeding pad (width of 4.4

m) was installed along the south end of the pens to help control mud around the bunks and water troughs. The remainder of the pen is dirt floor.

Air Sampling

As previously stated, the methane barn has two nearly identical pens in which air is exchanged using a negative-pressure ventilation system, meaning the fans pull air through the chambers rather than pushing it through them. The negative-pressure system minimizes loss of produced gases from the chamber via uncontrolled routes. By forcing the air to travel across the full length of the pens and mixing it along the way, gas concentrations of air near the fans can be made to accurately represent gas concentrations.

There are three separate sampling points, one within each pen and one outside of the south wall of the building to get an ambient sample. Each sampling port is located roughly 3 m above ground, and half that distance away from the wall (Figure 2A). Cotton cloth wrapped around a cage assembly acts as a pre-filter to keep most dust, debris, insects, etc. from entering the neoprene-tube sampling line (9.5 mm diameter Tygon, Grainger Industrial Supply). In each of the three sampling lines, a diaphragm pump (Thomas 2107 Series, Gardner Denver, Milwaukee, WI) pulls air through a short (~ 4 m) section of tubing at over 24 L/min, and travels down a 15 m length of sampling line to a solenoid. Roughly 25 L/min of airflow through the line is desired to flush the container (14.3 cm dia. x 82.8 cm) housing the methane analyzer in 30 seconds. Positive pressure is crucial for the sampling line because any small leaks are outward rather than fresh air coming inward – therefore, the air sample is not diluted (Judy, 2017). Each sampling line

is the same length to eliminate variation in emissions measurements that could occur with differing lengths of sampling line. The neoprene tubing is surrounded by 1.9 cm diameter PEX pipe (SharkBite, Grainger Industrial Supply) that protects the more fragile tubing and keeps it from crimping.

The solenoids (Figure 2B) are controlled (CR-6 Measurement and Control Datalogger, Campbell Scientific, Logan, UT) to direct the air either through a manifold (6-Station Stainless Steel Manifold, Pneumadyne Inc., Plymouth, MN) and another 21 m of main sampling line to the gas analyzers or out of the building. Ball valves in each sampling line allow for manual control. After some trial-and-error adjustment of an initial plan, a functional strategy was to set the solenoids on a timed rotation that proceeds in this sequence (Figure 3):

Ambient line for 2 minutes (flush only) → Pen 1 for 6 minutes (sample) →
Ambient line for 6 minutes (sample) → Pen 2 for 6 minutes (sample) → Repeat

With this sequence, there is only one line of air being delivered to the analyzers, analyzers are flushed with ambient air between receiving air from the chambers, and there is a reasonable compromise between sampling duration and frequency of sampling (three 20-minute cycles per hour). There is also a clear indication in the data of when a new cycle begins. We learned from experience not to be reliant on recorded times to identify sample sources, especially in a field setting like this one, where a host of issues can lead to missing or mismatched clock times.

Once the sample airstream enters the analysis room (Figure 4), it passes through a compressed air filter (150 psi Compact Compressed Air Filter, Wilkerson, Richland, MI). Then the airstream is split into two paths, one heading to a flow meter prior to entering the methane analyzer, and one heading to a flow meter prior to entering the carbon dioxide analyzer. The flow meters (2500 Series Acrylic Tube Variable Area Flowmeter, Brooks Instrument, Hatfield, PA) were used to manually regulate the amount of air simultaneously passing through the analyzers. The container volume for the methane analyzer is almost fifteen times larger than for the carbon dioxide analyzer, which means flush times would differ greatly for the same airflow rate. Therefore, airflow rates were regulated (restricted in the CO_2 line) to achieve similar flush times. Since only gas concentrations and air properties are needed from the air sampling and analysis system, no attempt was made to continuously monitor airflow rate through the system – routine system checks only by checking the flow meters

Gas Analysis

The gas analyzers used in this system are a methane analyzer (LI-7700 Open-Path CH_4 Analyzer) and a carbon dioxide analyzer (LI-7500DS Open-Path CO_2/H_2O Analyzer; LI-COR Biosciences, Lincoln, NE). The methane analyzer operates using near-infrared laser and wavelength modulation spectrometry to detect the absorption of methane in the air sample. The resolution of this instrument is 5 ppb RMS noise at 10 Hz, in typical ambient concentrations (2 ppm CH_4). The measurement frequency of this analyzer is sub-MHz, meaning absorption can be detected at levels smaller than 10^{-5}. The carbon dioxide analyzer uses non-dispersive infrared spectroscopy to measure carbon

dioxide and water densities in the air sample. The resolution of this instrument is 0.11 ppm RMS noise at 10 Hz.

Each analyzer is enclosed within an airtight cylindrical case made from PVC pipe and made airtight by using rubber on each end, with inlet and outlet ports for moving the sample airstream through the case. The air volume in the methane analyzer case is 13.35 L compared to 1 L in the carbon dioxide analyzer case. The design criterion for airflow rate was to obtain a complete flush of the analyzers and the main sampling line within the first 30 seconds of a sampling period. This resulted in a design airflow rate of 32 L/min (16 L flush in 30 seconds).

Calculation of Emission Rate

Given a system flow rate of at least 32 L per minute, the first 30 seconds of any sample period represents a transition period between samples, when the sampling line and analyzer cases are being flushed. In processing the data, whenever the source for sampling switches from a pen to the ambient air, or vice versa, the first 30 seconds of measurements need to be omitted. A 5.5-minute average measurement is then used for each sampling phase of the sequence, while the 2-minute ambient period is used strictly to flush the system between pens and to allow for easy determination of when the sampling cycle resets. Measurements are collected every second by the analyzer and then exported to data storage. The data are manually cleaved to begin at the first 2-minute ambient cycle at the beginning of each day (starting at midnight). The 2-minute start to each data file was selected manually using the software provided with the methane and carbon dioxide analyzers. Any data that was interfered with (cleaning the pens with a

skid steer, feeding doors being left open) were discarded manually by using the same software. To discard data, it must line up with the correct times in the 20-minute cycle as to not get out of line for when it is further analyzed in a script. Data are then run through a script written using R (The R Project for Statistical Computing) to obtain average concentrations (ppm) for the 5.5-minute sampling periods. These average short-term concentrations are then compiled into daily values and those means (ppm) are converted to grams per head per day using the following equations:

Water Vapor Flux:

[1] $F = \frac{U_o \cdot (w_o - w_i)}{1 - \frac{[w_{MF}]}{1000}}$

F: water vapor flux from the pen, mmol/min

U_o: exhaust air flow rate from the pen, m^3/min

w_o, w_i, water vapor density in outgoing (pen) and incoming (ambient) airstreams, respectively, mmol/m^-

[w_{MF}]: water vapor mole fraction in incoming airstream, mmol/mol.

CO_2 Emission Rate:

[2] $m_{CO2} = U_o \cdot (CO_{2-o} - CO_{2-i}) + \frac{F \cdot [CO_{2\ in}]}{1000 \cdot 1000}$

m_{CO2}: CO_2 emission rate from pen, mmol/min

U_o: exhaust air flow rate from the pen, m^3/min

$CO_{2\text{-}o}$, $CO_{2\text{-}i}$: CO_2 densities in outgoing and incoming (ambient) airstreams, mmol/m

F: water vapor flux, mmol/min

[$CO_{2\ in}$]: CO_2 mole fraction in incoming airstream (μmol/mol)

CH_4 Emission Rate:

[3] $m_{CH4} = U_o \cdot (CH_{4-o} - CH_{4-i}) + \frac{F \cdot [CH_4\ in\ ]}{1000 \cdot 1000}$

m_{CH4}: CH_4 emission rate from pen, mmol/min

U_o: exhaust air flow rate from the pen, m^3/min

$CH_{4\text{-}o}$, $CH_{4\text{-}i}$: CH_4 densities in outgoing and incoming (ambient) airstreams, mmol/m^3

F: water vapor flux, mmol/min

[$CH_{4\ in}$]: CH_4 mole fraction in incoming air stream (μmol/mol)

The following equations were used to convert the emission rates obtained for methane and carbon dioxide from mmol/minute into kg/d:

CH_4 kg/pen/d = (m_{CH4} * 0.016[1])/0.6944[2]

CO_2 kg/pen/d = (m_{CO2} * 0.044[1])/0.6944[2]

[1]0.016 and 0.044 are the molecular weights of CH_4 and CO_2, respectively, in kg

[2]0.6944 = conversion factor for grams/minute to kg/day

Trial Setup

The first trial with cattle was done to validate the methane barn system. Cattle in this first trial (described in Chapter III in more detail) were fed the same diet at two different levels of intake because intake is known to be a main factor in determining the total amount of methane produced per day (Blaxter and Clapperton, 1965). The results obtained were in line with what was expected relative to the differences in CH_4 production (g/d) between intake levels and gave confidence that the system was functioning as intended. After the completion of two trials through the methane barn, the ambient carbon dioxide and methane concentrations measured by the analyzers were compared to expected ambient concentrations. Using 106 d of ambient collections,

average ambient methane was 2.28 (SD = 0.23) ppm and average ambient carbon dioxide was 415(SD = 13.5) ppm. In 2016, atmospheric ambient concentrations of carbon dioxide and methane were recorded in California and Hawaii, respectively. Carbon dioxide and methane were 408 ppm and 1.90 ppm respectively, which is slightly lower than what was observed by the analyzers detected at the methane barn (Prinn et al., 2016; Dlugokencky and Tans, 2016).

During trials, there are more groups of cattle (experimental units) than there are study pens, so the groups are rotated through the pens. The procedure put in place is that two groups of cattle are moved into the methane barn each week, with remaining cattle groups being held in outdoor pens. Cattle stay in the methane barn for a period of 5 consecutive days, during which emissions data for each pen are collected for use in assessing treatment effects. Then, these cattle groups are moved to outdoor pens and measurements continue in the chambers for another day to monitor emissions from just the manure that was deposited by the cattle in the pens over the previous five days. Following that, manure is cleaned out using a skid loader and then the pens sit empty for that day to establish baseline measurements for each pen prior to the next two groups of cattle entering the facility. The effect of manure on methane (ppm) in the chamber when cattle are not present compared to when both the manure and cattle are not present in the chamber is illustrated in Figure 5.

Discussion

There are positives and negatives to the different collection methods used for quantifying methane production from beef cattle. Headboxes are the most common

method, operating under a negative-pressure system that withdraws a known flow rate of air containing enteric and respired gases. The gas emissions are used to indirectly measure the heat produced by the animal and can be used to determine energy utilization. According to Murray et al. (1976), only 13% of methane emissions come from the hindgut portion, and 89% of hindgut methane production is respired through the lungs, so the headbox method may only miss a small amount of the total animal emissions, but is nonetheless a limitation to this method. Hellwing et al. (2012) described a collection method using four whole-body chambers that were placed in a tie-stall barn, which provided visual contact with other cows to help alleviate some of the isolation stressors. Whole-body chambers account for hindgut and fecal methane production, which enables more accurate complete animal emission measurements. However, whole-body chambers are more expensive and less mobile than a headbox, and can present some of the same issues related to animal stress, including DMI depression, limited movement, and isolation from other animals, which can alter methane production.

There are some examples of production-scale chambers that are able to collect from multiple animals at the same time by applying respiration calorimetry to current livestock buildings with ventilation systems in place (Kinsman et al., 1995; Tremblay and Masse, 2008; Bluteau et al., 2009). A positive of this design is that methane production is collected from both enteric fermentation as well as hindgut (including manure), but the design is limited by not being able to distinguish between the two sources. Taking methane measurements on a herd-scale is beneficial because it represents industry practices and can eliminate some animal stressors that are observed in other collection

methods. The animals are not isolated or restrained so behavior should not be impacted using this method (Tremblay and Masse, 2016).

In the methane barn the animals are free to behave as they would in the outdoor pens, as the indoor and outdoor pens have similar pen layouts, use the same models of feed bunks and waterers, and have a similar concrete apron leading up to the bunks. Similar to the other production-scale methods, the methane barn allows for detection of effects of feed quality, intake and composition on methane production. However, a system like this presents challenges to measuring the ventilation rate relative to single-animal methods due to the larger scales of airflow and because buildings are not as airtight (Tremblay and Masse, 2016). In the methane barn, it was estimated that 90% of air entering the pens comes through the south side, as evidenced by enough negative pressure being created that the gravity air inlets open when the fans are running. Prior to sealing up the walls and around the sliding alley doors, the gravity air inlets would not open at all with both fans running, implying that a significant amount of airflow was previously entering through leaks in the building. The main negative effect of leakage is that air may enter and exit a chamber without traversing the animal space. So, as leakage increases, the potential increases for gases to collect in the animal space and not be fully represented in the exhaust air. Facility maintenance during and between trials is important to control leakage.

Tremblay and Masse (2008) concluded that although there are many benefits to these production-scale calorimeters, they lack in precision relative to a respiration chamber. These same authors concluded that to get the best measurements from a setting

like cattle on pasture or in a feedlot, open-path lasers are the most appropriate method for determining combined manure and enteric emissions. However, open-path lasers may not be the most appropriate for use in livestock buildings due to the effects of other farm buildings, manure piles, and other obstacles to even wind movement, and wind direction. Typically, open-path measurements require recording wind speed, direction and proximity to the herd (Tremblay and Masse, 2016) in order to determine air velocity profiles and resulting emissions. However, in the methane barn, measurement of the ventilation rate through the exhaust fans provides the necessary airflow information for calculating emission rates, eliminating the need to continuously record air speeds past the analyzers or in other locations.

The fans in the methane barn are run at full speed to remove a variable and make the analyses easier with constant ventilation rate. No efforts are employed to control temperature in the facility during cold weather – and temperatures indoors generally track those outdoors. A couple of lessons learned from operating the methane barn during cold weather included that chamber ventilation rate needs to match cattle condition and the measurement ranges of the analyzers. During the first winter of use, only the smaller fan was run – providing more than the standard minimum air exchange needed during cold outdoor conditions. However, cattle brought in from outdoor pens had acclimated to colder temperatures (i.e. thick hair coats) and, as noted earlier, showed some signs of heat stress at temperatures near and below 10 C. Also, methane and carbon dioxide concentrations increased with the reduced ventilation rate, causing plateaus and gaps in the data. These anomalies were later determined to be caused by the concentration

exceeding the upper measurement threshold of the analyzer. Running both fans at all times during trials addresses these concerns.

Conclusion

Enteric methane production from beef cattle has become an important topic due to the environmental concerns associated with it. Methane production is not only an environmental concern, but an energetic loss to the animal as well, so researching ways to mitigate methane production from cattle could lead to less environmental impacts as well as more energy available for animal performance and production. In order to measure ways to mitigate methane, robust methods must be used that are applicable to the industry, otherwise the research may not be viewed as applicable for many producers. The methane barn was constructed with producers in mind, as well as to monitor gases on a more robust level in a less stressful environment for the cattle. The methane barn is capable of detecting differences in methane production between pens and is a viable option for evaluating mitigation strategies using larger numbers of animals at the same time.

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Figure 1: Methane barn side-by-side chamber and sampling system design

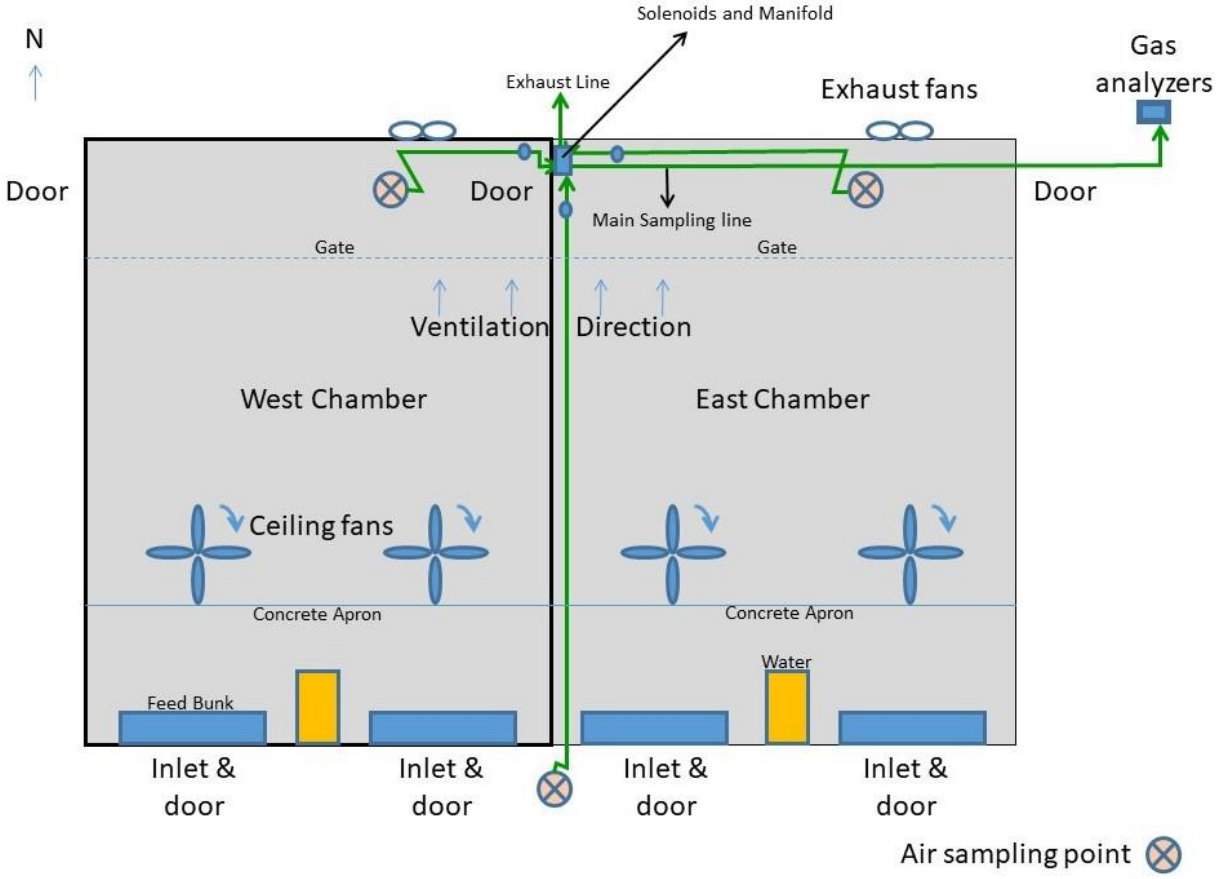
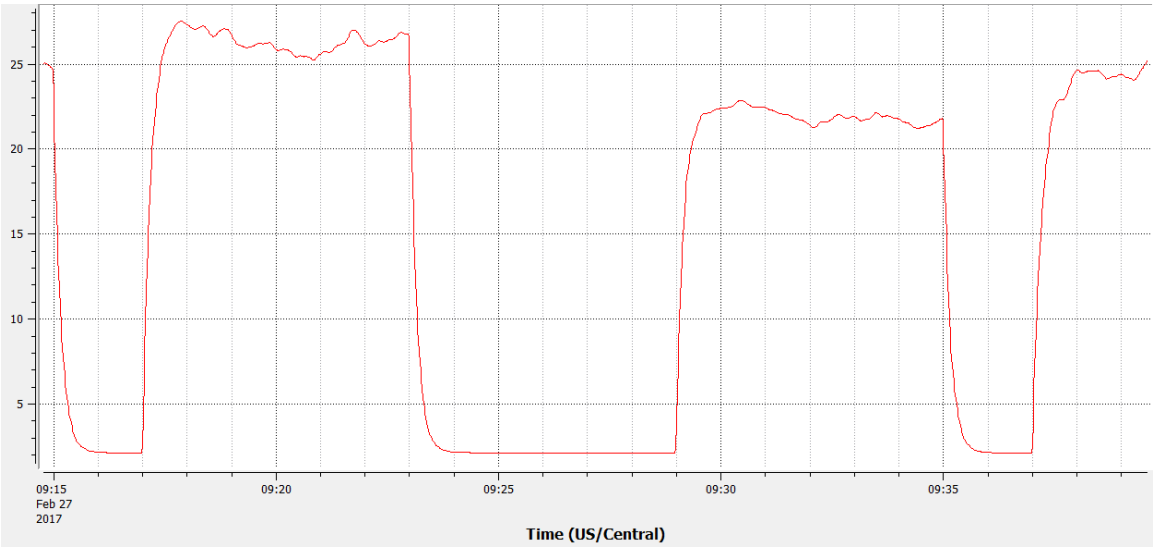


Figure 2A: North wall of the methane chamber, showing the two exhaust fans that create the negative pressure system, the sampling point above the fans, and the diaphragm pump used to push air along the sampling line.

Figure 2B: Central wall between the chambers showing the 3 solenoids, manifold, exhaust line (upper right corner), and main sampling line, attached to the manifold.

Figure 3: 20-minute air sampling cycle



Description: Methane (ppm) on the Y-axis. 20-minute sampling cycle rotating between ambient line (2 minutes), chamber one (6 minutes), ambient line (6 minutes) and chamber two (6 minutes). Illustrates that only 30 seconds is needed to flush between sampling lines. Data were analyzed using 6 minute cycles excluding the first 30 seconds while the gas acclimates to the solenoid switching.

Figure 4: Gas analysis room where the methane and carbon dioxide analyzers are located. They are enclosed in the cylindrical casings designed for each analyzer. The air sample enters (top of picture) and passes through the air filter and flow meters prior to being analyzed.

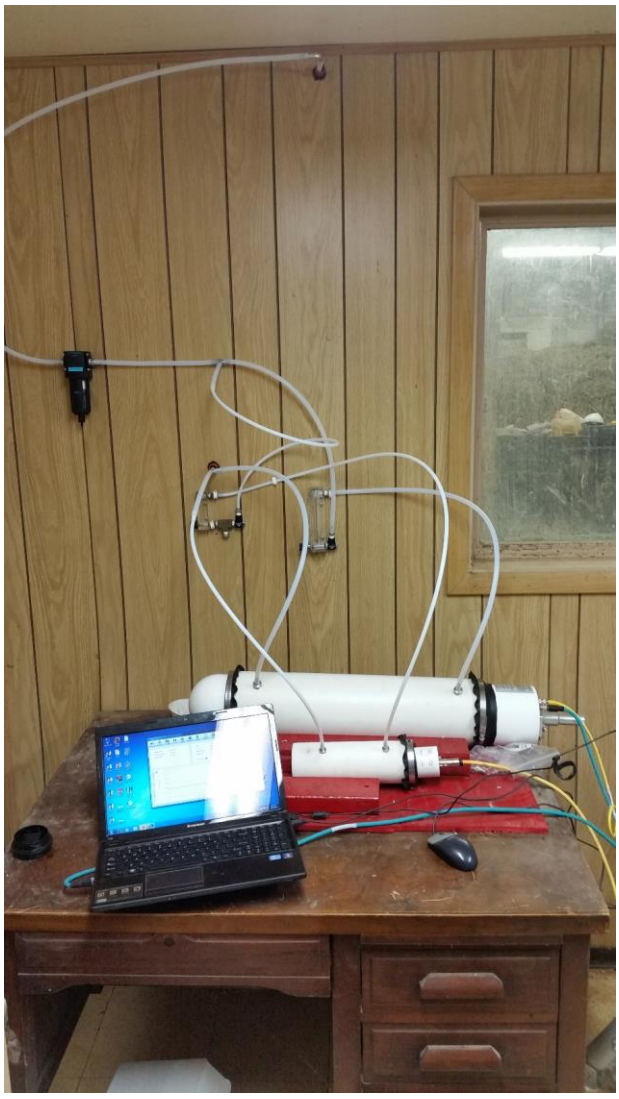
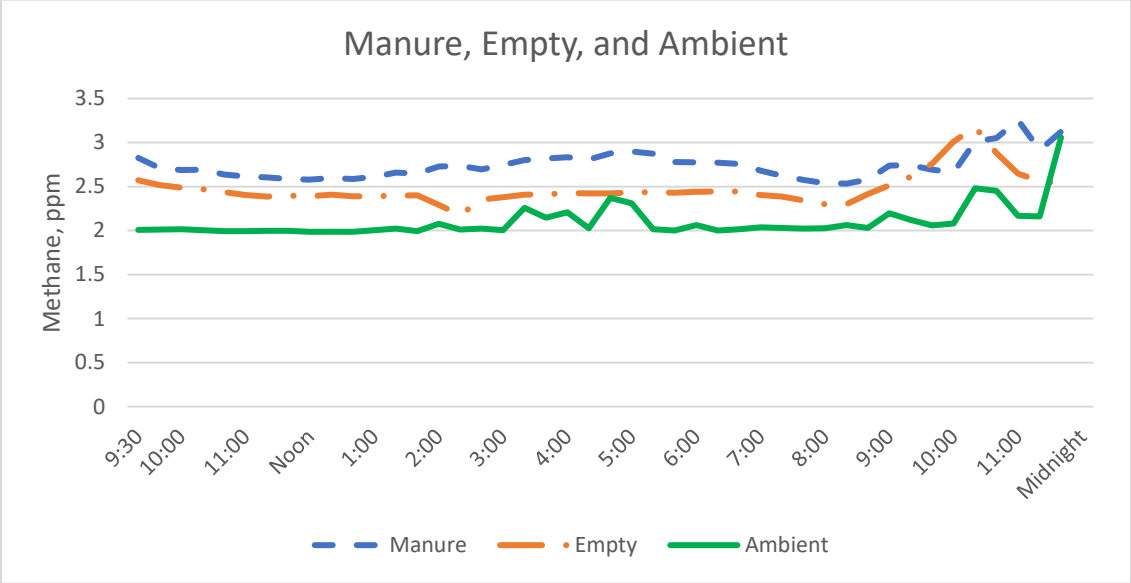


Figure 5: Manure in the chambers vs. no manure in the chambers



Description: Illustrates the effect that manure has on methane in the barn when no cattle are present compared to the empty chamber when no cattle or manure are present. Data are shown from 9 am to midnight. This period was chosen based off when the manure was cleaned for the empty chamber. The manure and ambient data come from 6/6/17 and the empty chamber data comes from 6/7/17.

CHAPTER III. Evaluation of methane production manipulated by level of intake in growing cattle and corn oil in finishing cattle.

T.M. Winders, B.M. Boyd, F.H. Hilscher, R.R. Stowell, S.C. Fernando, G.E. Erickson,

Department of Animal Science, University of Nebraska, Lincoln, NE 68583-0908

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[2]Corresponding author: C220 Animal Science Building, Phone: 402-472-6401; Fax: 402-472-6362; email: gerickson4@unl.edu

Abstract

Crossbred steers (n = 80, initial BW = 274 kg, SD = 21) were used to evaluate the effect of intake on methane production in growing steers. Two treatments with 4 pens per treatment (10 steers/pen) were evaluated in a generalized randomized block designed experiment, with BW as blocking factor. Treatments included feeding the same diet at two levels: *ad-libitum* or limit-fed at 75% of ad libitum. The diet consisted of 45% alfalfa, 30% sorghum silage, 22% modified distillers grains plus solubles and supplement at 3% on a DM basis. This trial was followed by a finishing trial (n=80; initial BW = 369 kg; SD = 25) to evaluate the effects of adding dietary corn oil on methane production. Two treatments with four pens per treatment (10 steers/pen) were used in a randomized complete block designed experiment. Cattle were re-randomized and blocked by BW and previous treatment. The treatments consisted of a control diet (CON) containing 66% corn, 15% wet distillers grains plus solubles, 15% corn silage, and 4% supplement. The corn oil treatment (OIL) displaced 3% corn by adding 3% corn oil. Methane was collected in 2 pen-scale chambers by collecting air samples continuously from pens by rotating every 6 minutes with an ambient sample taken between pen measurements. Steers fed ad-libitum had greater DMI by design and greater ADG ($P < 0.01$) compared to limit-fed cattle; however, efficiency of feed utilization was not different between treatments ($P = 0.40$). Cattle fed ad-libitum produced 156 g/d of methane, which was greater ($P < 0.01$) than limit-fed cattle (126 g/steer daily). In the finishing phase, body weight, gains, and carcass traits were not impacted by treatment ($P \geq 0.14$). Efficiency of feed use ($P = 0.02$) improved because intakes decreased ($P = 0.02$) by feeding OIL

compared to CON. Daily methane production was lower ($P = 0.03$) for the OIL fed cattle (115 g/steer daily) compared to the CON fed cattle (132 g/ steer daily) and there was a treatment by sampling period interaction ($P = 0.02$) due to magnitude of differences between OIL and CON across time. Methane was reduced ($P < 0.01$) by 17% for the OIL fed cattle compared to the control when expressed as methane g/kg of ADG. Feeding corn oil at 3% of diet DM reduced enteric methane production (g/d) by 15%, which was partially due to a 3% decrease in DMI.

Key Words: beef cattle, intake, methane, nutrition, oil

Introduction

Methane production from ruminant animals has been a focus in research studies due to the environmental concerns associated with rising levels of greenhouse gases (GHG). Ruminants, and especially beef cattle, have received attention due to the amount of methane they contribute to the global methane budget. Ruminants contribute 17% of the global methane production and beef cattle contribute 56% of the total CH_4 from ruminants (Conrad et al., 2009; Tubiello et al., 2013). The environmental concerns are a main reason that mitigation strategies are being pursued in cattle production, but the energetic loss to the animal associated with methane production is another reason for the research. The amount of energy lost as a result of methane production ranges from 2-12% with an average of 6% of energy intake lost from methane production (Johnson and Johnson, 1995).

One of the biggest determinants of methane production is the dry matter intake (DMI) of the animal. The diet digestibility, and subsequent profile of volatile fatty acid production plays a role, but amount of feed intake might be the biggest factor in how much methane is produced. Methane production is largely dependent on the quantity of feed intake, although it can be altered by the quality and digestibility (Blaxter and Clapperton, 1965). Forty-eight trials were examined by these authors and found that methane was increased as intake increased in all 48 instances.

Another strategy for methane mitigation is through lipid supplementation. Hales et al. (2017) described three ways that dietary lipids reduce methane: 1) biohydrogenation of fatty acids, 2) increased propionate production from lipolysis converting triglycerides

to glycerol, which is then converted to propionate by anaerovibrio lipolytica bacteria, and 3) reduction in available fermentable substrate in the rumen as fatty acids are not fermentable. Biohydrogenation converts unsaturated fatty acids to saturated fatty acids in the rumen, beginning with bacterial isomerase, which changes the conformation from cis to trans fatty acids. Reductases then remove the double bond, forming a saturated fatty acid. Altering the lipid profile from unsaturated to saturated is done to avoid microbe toxicity issues related to unsaturated fatty acids in the rumen (Jenkins et al., 2007). Biohydrogenation reduces methane as a hydrogen is needed, thereby competing with methanogens for lipid profile transformation. Biohydogenation is unable to compete at a high level with methanogens though, as only 1% of metabolic hydrogens are used for biohydrogenation, while 48% are used to reduce carbon dioxide to methane (Johnson and Johnson, 1995). Methanogens and protozoa struggle to survive in the presence of unsaturated fats, especially fats rich in lauric and myristic acids, which can lead to methane reduction (Dohme et al., 2000). Feeding fats at an inclusion of 6% dietary DM has been shown to reduce methane by 15%, but when fed in excess of 6-7% dietary DM can lead to a depression in feed intake Patra, 2013; Beauchemin et al., 2008). Patra (2013) showed that propionate can be increased with fat inclusion, probably due to less methanogens being present, allowing for a greater accumulation of hydrogens available for propionate production as well as the conversion of triglycerides to propionate through lipolysis. Overall, intake level is the main driver behind methane production, and lipids are a viable mitigation option and will be discussed further throughout this chapter.

The objectives of these experiments were 1) evaluate the impact of restricting intake on methane production, and 2) evaluate the effect of corn oil supplementation in finishing diets on methane production

Materials and Methods

All animal care and management practices were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee.

Experiment 1:Growing diets

A 105-d growing study was conducted at the Eastern Nebraska Research and Extension Center feedlot near Mead, NE. Eighty steer calves (initial BW = 274 kg; SD = 21 kg) were utilized. Calves were received, weighed, and revaccinated against *infectious bovine rhinotracheitis* (IBR), bovine viral diarrhea types I and II, and parainfluenza type 3 (Bovi-Shield Gold 5, Zoetis Animal Health, Parsippany, NJ) and parasites (Dectomax, Zoetis Animal Health; (StandGuard, Elanco Animal Health, Greenfield, IN). Cattle were limit-fed a common diet of 50% alfalfa and 50% Sweet Bran (Cargill Corn Milling, Blair, NE) at 2% of BW for 5 d (to equalize gut fill) and weighed for 2 consecutive days, then averaged, in order to obtain an accurate initial BW (Watson et al., 2013). Steers were blocked by BW (n = 3), stratified within BW, and assigned randomly to pens. Pens were assigned randomly to treatment, with 10 steers/pen and 4 pens/treatment.

Treatments consisted of identical diets, either being fed ad-libitum or limit-fed. Diets were 45% alfalfa, 30% sorghum silage, 22% modified distillers grains plus solubles and 3% supplement on a DM basis (Table 3.1). The supplement was formulated to provide 26 mg/kg of monensin (Rumensin, Elanco Animal Health). The limit fed group

received 75% of the ad-libitum group in their respective replication within block DMI from the previous week. On d 1, steers were implanted with 100 mg trenbolone acetate and 14 mg estradiol benzoate (Synovex Choice, Zoetis Animal Health). Ending BW was obtained after 5 d of limit feeding the same initial diet of 50% alfalfa and 50% Sweet Bran (Cargill Corn Milling) and collecting BW on two consecutive days. The ending BW values account for the 5 d limit feeding by subtracting 0.454 kg/day from ending BW.

The steers were rotated through two pen-scale methane chambers with two side-by-side enclosed dry-lot pens sharing a middle wall. Gravity inlets on the south wall of the building allowed air to enter the chambers. Air is drawn through the inlets using two fans on the north wall, creating a negative pressure system. Air is pulled through each pen and exits through the fans, with a sampling line positioned above the fans. Fans were calibrated twice, once prior and once after the trials (FANS System, Iowa State University). Airflow through the chambers with two fans running was 1274 L/s. Air samples in each pen are pulled into the sampling line with a pump and brought through to a solenoid system controlled by a data logger. The solenoids switch sampling between the ambient line, pen 1, and pen 2, allowing for each pen to be sampled for 6 minutes of each 20-minute cycle.

A 2-minute ambient sampling allows for easy recognition of when the cycle resets when data were being analyzed, as pen 1 always follows the two-minute sampling period. The 6 minute ambient time allows for the system to be flushed between pen 1 and pen 2 sampling periods and provide ambient concentrations of carbon dioxide and methane.

Emissions data are averaged across each 6 minute time point, excluding the first 30 seconds to avoid including lower measurements as the gas acclimates to the solenoid switching. Gas production per day is an average of all of the 6 minute averages per pen per day. Cattle were in the methane barn for five consecutive days, then removed and manure measurements were taken for 1 d, and then manure was removed and clean pens were measured for 1 d. With eight pens of cattle, and two pens in the methane barn, it allowed for cattle to enter the barn for one 5 d period every 4 weeks. Each treatment was represented at all times in the methane barn, as each block replication had emission collections at the same time. Each pen had three 5 d collection periods throughout this trial, and pens were alternated between pen 1 and pen 2 each rotation through the methane barn. Gas measurement sampling errors on the first two collection periods made those data unusable. The error was a result of the methane analyzer being saturated, as the upper limit for accurate measurements is 50 ppm and at certain times throughout the day this limit was exceeded. The problem was corrected for the third collection period by using two fans per pen instead of one, which moved air through at a faster rate and decreased methane concentration in air samples. Only the third collection period emissions data are shown.

The gas analyzers used in this system are a methane analyzer (LI-7700 Open-Path CH_4 Analyzer) and a carbon dioxide analyzer (LI-7500DS Open-Path CO_2/H_2O Analyzer; LI-COR Biosciences, Lincoln, NE). The methane analyzer operates using near-infrared laser and wavelength modulation spectrometry to detect the absorption of methane in the air sample. The resolution of this instrument is 5 ppb RMS noise at 10

Hz, in typical ambient concentrations (2 ppm CH_4). The measurement frequency of this analyzer is sub-MHz, meaning absorption can be detected at levels smaller than 10^{-5}. The carbon dioxide analyzer uses non-dispersive infrared spectroscopy to measure carbon dioxide and water densities in the air sample. Methane and carbon dioxide production were calculated using the equations referenced in chapter II. Methane (g/kg DMI) was calculated using DMI across the 105 d growing period as well as DMI observed in the methane chambers. Dry matter intakes were not different when fed in the outdoor pens compared to in the chambers but were more variable.

Data were analyzed using the MIXED procedure of SAS as a generalized randomized block design with three blocks; blocks 1 and 3 had one replication, while block two had two replications. Pen was considered the experimental unit and BW block was included in the model as a fixed effect. Using the R script to cleave the first 30 seconds of each 6 minute sample results in 8.3% of the data not used. The data summarized accounted for 93.0% of the data due to periodic gas analyzer errors resulting in data not being collected.

Experiment 2: Finishing diets

A 127-d finishing study was conducted at the Eastern Nebraska Research and Extension Center feedlot near Mead, NE. Crossbred steers (n = 80; initial BW = 369 kg; SD = 25 kg) were utilized. The cattle were used on Exp. 1 prior starting this finishing trial. Cattle were limit-fed a common diet of 50% alfalfa and 50% Sweet Bran (Cargill Corn Milling, Blair, NE) at 2% of BW for 5 d (to equalize gut fill) and weighed two

consecutive days, then averaged, in order to obtain an accurate initial BW (Watson et al., 2013). Steers were blocked by BW and by previous treatment (ad-libitum or limit-fed), stratified within BW block, and assigned randomly to pens. Pens were assigned randomly to treatment with 10 steers/pen and 4 pens/treatment.

The control diet was a 50:50 blend of high-moisture corn (HMC) and dry-rolled corn (DRC) at 63%, wet distillers grains plus solubles at 15%, corn silage at 15% and supplement at 4% (DM basis). The treatment diet included 3% corn oil displacing 3% of the HMC:DRC blend with the rest of the diet being identical to the control (Table 3.2) The corn oil was sourced from an ethanol plant that extracted the oil in the process of producing ethanol (E Energy Adams, Adams NE). Cattle were adapted to the finishing diet over a 24-d step-up period. Wet distillers grains plus solubles was held constant at 15% while corn silage started at 81% and was displaced by HMC:DRC blend down to 15% over this period. Corn oil was introduced to the corn oil diet on d 18 of the step-up period and displaced 3% of the corn silage. The supplement was formulated to provide 33 mg/kg of Rumensin (Elanco Animal Health) and 9.7 mg/kg of Tylan (Elanco Animal Health). Urea was added to both diets at 0.5% of diet DM to ensure rumen degradable protein requirements were met. On d 1 cattle were implanted with 100 mg trenbolone acetate and 14 mg estradiol benzoate (Synovex Choice, Zoetis Animal Health). The steers were harvested on d 128 at Greater Omaha (Omaha, NE). Hot carcass weight and liver abscesses were recorded during harvest, and a dressing percent of 63% was used to calculate final BW. The carcass was chilled for 48 hours and fat thickness, LM area, and USDA marbling scores were recorded and yield grade was calculated.

The same pen-scale methane calorimeter chamber described for Exp. 1 was used for this trial. The cattle were brought through the methane barn for 3 periods, which lasted for 5-d of continuous collections each period. Each treatment was represented in the methane barn at all times, as each blocks' replication entered at the same time, for a total of two pens being collected at the same time. The pens were alternated between pen 1 and pen 2 each time they entered for the sampling period to remove any bias from the methane chambers.. Feed refusals were removed from the bunks once a week (at the end of each 5 d period) and weighed. A 59.9% diet DM was used to calculated dry feed refusals to correct intakes accordingly while the cattle where in the methane barn.

Data were analyzed using the MIXED procedure in SAS as a randomized complete block design with all blocks (n=4) having one replication. Pen was the experimental unit and BW block was treated as a fixed effect. Gas production data were gathered over three periods, so the data were analyzed using repeated measures using compound symmetry or first order autoregressive covariance structures, chosen from the lowest fit statistic values. Treatment, period, and block were included in the model as fixed effects. Treatment by period interactions were tested for methane production across time. Methane and carbon dioxide values were calculated the same was as described in experiment 1. Using the R script to cleave the first 30 seconds of each 6 minute sample results in 8.3% of the data not used. The data summarized accounted for 93.2% of the data due to periodic gas analyzer errors resulting in data not being collected.

Results and Discussion

Experiment 1

Performance

Dry matter intake and average daily gain were lower ($P < 0.01$) for the limit-fed cattle compared to the ad-libitum cattle, while no difference ($P = 0.40$) was observed for feed efficiency (Table 3.3). Observing no difference in feed efficiency differs from what Plegge (1987) and Hicks et al. (1990) observed. Hicks et al. (1990) reported restricting DMI at 85% of ad-libitum can lead to improvements in feed efficiency. However, Murphy and Loerch (1994) reported that cattle limit-fed 90 and 80% of ad-libitum intake on corn-silage based growing diets had a linear reduction ($P < 0.01$) in ADG, but did not observe a difference in feed efficiency. Other authors (Hanke et al. 1987; Wagner et al. 1987) have shown no difference in feed efficiency similar to what was observed in this trial. A 24% reduction in ADG observed in this trial is greater than the 15% reduction reported by Murphy and Loerch (1994) when cattle were restricted to 80% of ad-libitum intake. The 24% reduction observed in this trial is proportionate to the percent of feed withheld from the limit-fed cattle (25%), implying that with every unit of feed withheld, no improvement in feed efficiency occurred. Glimp et al. (1989) reported a 15% increase in ADG when limit feeding lambs to 89% of the ad-libitum diet. In finishing diets fed to steers, Hicks et al. (1990) observed a 7% reduction in ADG when limited to 85% of the ad-libitum group. Murphy and Loerch (1994) reported a 12% reduction in ADG for cattle fed a finishing diet restricted to 90% of ad-libitum intake. Mertens (1994) states that one of the most important aspects to forage quality in relation to animal performance is the level of intake. The improvements in feed efficiency that some authors have reported are likely from an increased diet digestibility, reduced animal movement, and a

reduction in organ size as an animal is fed closer to maintenance requirements (Hicks et al., 1990). Most variation associated with dry matter digestibility (DMD) and digestible energy (DE) intake is associated with differences in intake level (60-90%), whereas only 10-40% is associated with the digestibility of the feed (Mertens, 1994). Dry matter intake while in the methane barn during the period was reduced ($P < 0.01$) for the limit-fed cattle as well; consistent to when cattle were in outdoor pens. Ending BW was greater ($P < 0.01$) for the ad-libitum cattle compared to limit-fed cattle. The efficiency response observed in other trials related to limit-feeding (Plegge, 1987; Hicks, 1990) was not observed in this trial. This could be due to not approaching maintenance levels, as the steers were still fed above maintenance.

Methane

Methane production (g/d) was greater ($P < 0.01$) from the ad-libitum cattle compared to the limit-fed cattle (Table 3.4). The ad-libitum cattle produced 20% more methane per day. This is similar to the results that Blaxter and Clapperton (1965) observed when 48 sheep trials were analyzed to determine the relationship between intake and methane production. These authors reported that total daily methane produced increased in all 48 cases as level of intake increased. Beauchemin and McGinn (2006a) evaluated the effect of intake level on methane production in high-forage and high-concentrate diets. These authors found that methane production (g/d) was greater ($P < 0.01$) for ad-libitum cattle compared to cattle restricted to 65% of the ad-libitum intake. In the present study, methane production per kg DMI over the 105 days tended ($P = 0.07$) to be 8.0% lower for the ad-libitum group compared to the limit-fed group. Beauchemin

and McGinn (2006a) reported that methane production (g/kg DMI) was not different between ad-libitum and limit-fed treatments. This is similar to what Blaxter and Clapperton (1965) observed for growing diets (low energy) when they reported that increasing feed amounts had no effect on methane production as a percent of intake. However, in finishing diets (high energy), these authors reported that as feeding level increased, methane as a percent of intake is reduced. Johnson and Johnson (1995) found that as feeding level increased, methane lost as a % of GEI decreased by 1.6% for every unit of intake increased. The reason that Blaxter and Clapperton (1965) saw the difference in methane production between low quality and high quality diets could be a result of passage rate and intake. Increasing the intake of high forage diets has less impact on passage rate than increasing intake on high concentrate diets (Mathison et al., 1998). This could be a result of concentrates typically having smaller particle sizes to begin with, enabling passage rate to increase. Methane production per kg DMI during the chamber measurement period was not different ($P = 0.53$) between ad-libitum and limit-fed treatments. Methane per kg of ADG was not different ($P = 0.41$) between treatments. This would be expected after observing no improvement in efficiency for the limit-fed cattle because the same diet was applied to both treatments. The proportional reduction in methane observed from limit feeding is a result of less fermentable substrate entering the rumen, leading to less fermentation, and therefore less methane production. Dry matter intake was restricted by 25% for the limit-fed cattle and methane (g/d) was reduced ($P < 0.01$) by 19% compared to the ad-libitum treatment. This is similar to the conclusion made by Beauchemin and McGinn (2006a) when they reported that the

reduction in methane was proportional to the reduction in intake, because on a methane g/kg DMI basis, there were no differences between treatments.

Carbon dioxide

Carbon dioxide (g/d) was lower ($P = 0.04$) for the limit-fed cattle compared to the ad-libitum cattle (Table 3.4). Carbon dioxide (g/kg DMI) over the 105 d growing trial was lower ($P = 0.02$) for the limit-fed cattle compared to the ad-libitum group, but when analyzed relative to intake during the sampling period, carbon dioxide production was not different ($P = 0.16$). Carbon dioxide per kg of ADG was not different ($P = 0.11$) between treatments, although it was numerically 16% greater for the limit-fed group compared to the ad-libitum group. The ratio of CH_4:CO_2 was significantly lower ($P = 0.02$) for the limit-fed cattle compared to the ad-libitum group. This implies that the limit fed group produced less methane in proportion to carbon dioxide than the ad-libitum group, and could be theorized that the limit fed group should have been more efficient because of it. This was not observed though, as limit-fed cattle feed efficiency was not different from the ad-libitum cattle. Pesta et al. (2015) showed a significant ratio difference ($P < 0.01$) between cattle on a low quality forage diet either with or without monensin present, with the monensin treatment reducing the ratio of methane relative to carbon dioxide. However, these same authors found no difference with the same treatments on a high quality forage diet. The impact that intake has on methane production is well documented and understood, with these results confirming that level of intake is a main mechanism driving variation in methane production.

Manure

Methane and carbon dioxide emissions from the manure were measured for one day after the cattle completed their 5 d collection period. The measurements are from the accumulation of five days of manure building up in the chamber. Following one day of manure measurements, the manure was removed and chambers were measured for another day to obtain a baseline level of emissions. Methane from the manure was 0.20 g/steer daily (SD = 0.25), while carbon dioxide was 114 g/steer daily (SD = 67). Murray et al. (1976) reported that 13% of methane production comes from the hindgut, while the rest is expired through the lungs or eructation from the rumen. Of the 13% methane produced in the hindgut, 89% is respired through the lungs, leaving less than 1.5% of total emissions coming from the rectum as compared to the mouth. The results in this trial would suggest less than 2% of daily methane emissions are from the manure. This could be a result of continuous methane release from the manure over the 5 d period, resulting in less volatiles being released on d 6 (during the measurement period) than when first excreted from the animal. Cattle were not present while manure emissions were being measured. This results in the manure being idle, which could also reduce emissions.

Baseline carbon dioxide levels were 325 g/steer daily when manure and cattle were removed from the chambers and is contributing to the carbon dioxide measurements observed with manure and cattle in the chamber. Baseline methane levels are 0.14 g/steer daily when manure and cattle are removed from the chamber, which contributes to the methane measurements reported with manure and cattle in the chamber. Emissions from manure were calculated by taking the levels recorded with manure in the chamber and subtracting the baseline levels recorded after manure removal from the chamber. The

methane and carbon dioxide production from manure appears to be negligible but may be underestimated with these methods.

Experiment 2

Performance

Initial BW and final BW were not different ($P \geq 0.39$), while DMI was reduced ($P = 0.02$) for cattle fed 3% corn oil compared to the control (Table 3.5). Pesta et al. (2015) reported when feeding corn oil at 3% of diet DM to steers on a finishing diet, no performance (DMI, ADG, G:F) differences were detected relative to the control. Hales et al. (2017) reported no DMI reduction ($P = 0.39$) when steers were fed corn oil at 0, 2, 4, and 6% of the diet DM displacing DRC. A reduction in DMI was expected because the 6% corn oil treatment had a dietary lipid content of 9%, which exceeds recommended values of 6-7% (Beauchemin et al., 2008), but was not observed. Vander Pol et al. (2009) evaluated the effects of corn oil inclusion at 0, 2.5, or 5% of the diet DM on finishing heifer performance and saw a tendency for reduced DMI of 10% for the 5% corn oil treatment compared to control. These results are similar to the current study where DMI was reduced by 4% with corn oil supplementation. However, Gillis et al. (2004) reported no reduction ($P = 0.23$) in DMI when feeding 4% corn oil to heifers on a finishing diet. Burhoop (2017) tested the effects of adding 2% corn oil back to a diet with 30% de-oiled MDGS and compared it to a control diet and a de-oiled MDGS diet. This author observed the corn oil treatment resulted in heavier ($P < 0.05$) final BW compared to the control and equal to the de-oiled MDGS treatment, however, DMI was reduced for the 2% corn oil treatment compared to the de-oiled MDGS treatment ($P < 0.05$). Gillis et al.

(2004) also reported no reduction ($P = 0.23$) in DMI when feeding 4% corn oil to heifers on a finishing diet.

Other oils have been evaluated for methane production in growing and finishing diets. Zinn and Shen (1996) reported a decrease ($P < 0.01$) in intake of 9% when feeding yellow grease at 5% of diet DM to Holstein steers on a steam-flaked barley finishing diet. This reduction is similar to Vander Pol et al. (2009) and greater than the present trial. Machmüller and Kreuzer (1999) also reported an 8% decrease in DMI when feeding coconut oil at 3.5% of diet DM to sheep on high-concentrate diets. McGinn et al. (2004) reported no reduction ($P = 0.11$) in intake when feeding sunflower oil at 5% diet DM to growing cattle, however, other sources of oil have been shown to depress DMI. Beauchemin and McGinn (2006b) reported a decrease ($P < 0.05$) in DMI when feeding canola oil at 4.6% of diet DM to growing cattle fed a high barley silage diet. In contrast to the current trial and the previous literature referenced, Beauchemin et al. (2009) observed an increase ($P < 0.05$) in DMI when feeding sunflower and canola oilseeds at 3.7% of diet DM to lactating dairy cows, although oilseeds are more protected which could have led to those results.

In the present trial, corn oil did not affect ($P = 0.14$) on ADG, although a numerical improvement of 3% was observed for the corn oil treatment. A numerical increase in ADG could be a response to greater energy intake for the cattle fed 3% corn oil. However, Vander Pol et al. (2009) reported that as corn oil inclusion increased, ADG decreased linearly ($P = 0.04$). The tendency for reduced DMI could have offset the additional energy in the diet from corn oil. In their study, the dietary lipid content was 9%

for the 5% corn oil treatment, which exceeds the recommended limit for dietary lipids and could be affecting ADG as a result by possibly hindering fiber digestion. Gillis et al. (2004) did not see an ADG improvement ($P = 0.23$) when including corn oil at 4% of the diet DM in a finishing diet. Similarly, Pavan et al. (2007) observed a tendency ($P = 0.09$) for ADG to improve as corn oil inclusion increased with grazing cattle. Bessa et al. (2005) reported that ADG was not impacted when 10% soybean oil was supplemented to lambs on both low- and high-concentrate diets. Gassman et al. (2000) reported that ADG was reduced ($P < 0.05$) for cattle fed 2.5% CLA compared to the control in a corn-based finishing diet. This could be a result of lower DMI, which was reduced ($P < 0.05$) by 20% in the CLA treatment.

Feed efficiency (G:F) was improved ($P = 0.02$) by 7% for the corn oil cattle over the control cattle, which is what is expected as ADG was not different but DMI was lower for the corn oil cattle. Similarly, Burhoop (2017) reported an 11% improvement in G:F when supplementing corn oil at 2% of diet DM relative to the control, but this could be due to being fed in combination with MDGS. When compared to the MDGS treatment without corn oil added, the corn oil treatment had a 5% greater feed efficiency ($P < 0.05$). Vander Pol et al. (2009) reported that as corn oil increased in the diet, a linear decline ($P = 0.10$) in feed efficiency occurred. These same authors tested corn oil at 3.4% diet DM vs. a control diet containing predominantly DRC. These authors found that ruminal starch digestibility was reduced ($P < 0.10$) in the corn oil diet, which could help explain the previous results where they concluded that corn oil linearly reduced feed efficiency in finishing heifers. Total starch digestion was numerically lower in their

study for the corn oil treatment and the authors concluded that supplemental oil may impede total tract starch digestion. However, Hales et al. (2017) found that starch digestibility was not impacted by corn oil inclusion.

Other oils apart from corn oil have been examined for their effects on feed efficiency. Beauchemin and McGinn (2006b) had similar findings as they showed that canola oil reduced ($P < 0.01$) intake, had no impact on ADG, and therefore had an 11% improvement in G:F compared to the control. Similarly, Bessa et al., (2005) found that ADG did not differ when soybean oil was fed in a concentrate diet relative to the control, but G:F was improved with oil inclusion when fed to lambs. Pavan et al. (2007) saw that when corn oil was supplemented at 1.5 g/kg of BW to steers on tall fescue, G:F improved by 36% over the control. However, supplementing oil does not always lead to an improvement in feed efficiency. Gillis et al., (2004) fed corn oil to heifers and saw no differences in DMI, ADG, or G:F when analyzed as the first 32 d on treatment or the last 28 days on treatment. Discrepancies between these results could be due to a number of things including oil amount, type, form, and base diet composition.

All carcass characteristics were similar between treatments in this trial ($P \geq 0.27$; Table 3.5). These results are in agreement with what Gillis et al. (2004) reported for heifers fed corn oil in a finishing ration. These authors reported no differences between corn oil at 4% diet DM and the control for HCW, LM area, marbling score, or fat thickness. Pesta et al. (2015) reported that carcass characteristics were not different for cattle fed 3% corn oil compared to the control. Average daily gain was numerically greater for the cattle fed 3% corn oil, but no differences were detected in HCW. This

could be a result of the cattle fed corn oil having numerically lower initial weights of 9 kg compared to the control. Contrary to what was observed in the present trial, Vander Pol et al. (2009) reported that HCW were 7% lighter for the cattle fed 5% corn oil, which could be a result of lower intake leading to lower gains. However, Burhoop (2017) reported a heavier HCW as well as greater backfat, for the corn oil cattle compared to the control ($P < 0.05$), but no differences relative to the MDGS treatment without corn oil added. This implies that the HCW difference reported between the corn oil plus MDGS treatment is largely due to MDGS rather than corn oil, although the corn oil cattle were more efficient which is similar to the results observed in the present study. Pavan et al. (2007) reported some carcass parameter differences between control and corn oil supplemented steers that were finished on a forage-based tall fescue diet. These authors reported a linear improvement ($P = 0.01$) in HCW as corn oil inclusion increased from 0 to 0.75, to 1.5 g/kg BW. These differences can be attributed to replacing low-energy feed (fescue) with high-energy feed (corn oil). Overall, performance and carcass measures vary in response to corn oil (or other oil) supplementation. The discrepancies could be due to effects of lipid on DMI and fiber digestion and subsequent performance impacts.

Methane

Methane production (g/d) was reduced ($P = 0.03$) by 13% with the inclusion of corn oil relative to the control diet (Table 3.6). This result could be from less fermentable feed entering the rumen, from the lipids having a toxic effect on certain bacteria, or from biohydrogenation acting as a hydrogen sink (Beauchemin et al., 2007). Hales et al. (2017) is the only other study that has examined the effects of corn oil on methane

production in finishing beef cattle. These authors fed corn oil at inclusions of 0, 2, 4, and 6% diet DM, displacing DRC. Methane was collected from eight steers using respiration chambers over a 24-hr collection period. Methane (g/d) was reduced linearly ($P < 0.01$) as inclusion of corn oil increased. These same authors also reported a linear decrease ($P < 0.01$) in methane as a percent of GEI, with 6% corn oil reducing methane by 34%. Of the three ways that lipids can reduce methane that were previously discussed, these authors attributed the reduction in methane to biohydrogenation. They concluded that if displacing fermentable substrate with lipids were the reason, a reduction in VFA would have been observed but was not. Biohydrogenation is a hydrogen sink, but typically only uses 1% of metabolic hydrogens available in the rumen, being out-competed by methanogens that use 48% of the hydrogens (Johnson and Johnson, 1995). Beauchemin et al. (2008) reported that a 1% increase in supplemented lipid will reduced methane (g/kg DMI) by 5.6%. Hales et al. (2017) showed that for every 1% increase in supplemented lipid, methane (g/kg DMI) was reduced by 4.5%. In the present trial, methane (g/kg DMI) was reduced by 4.3% for every 1% increase in supplemented lipid.

Other oils have been examined for their effects on methane production. Beauchemin and McGinn (2006b) reported that canola oil reduced ($P < 0.05$) methane production (g/d) in heifers on a growing diet when fed at 4.6% of the diet compared to the control diet. Similarily, Machmüller and Kruezer (1999) fed 0, 3.5, and 7% inclusion of coconut oil to lambs. Inclusion of 3.5 and 7% reduced ($P < 0.01$) methane production by 28 and 73% respectively. The 73% reduction is larger than expected, but a reduction in DMI observed at this inclusion helps explain some of the reduction in methane, as well

as the influence of coconut oil on methanogens. Beauchemin et al. (2009) reported a reduction ($P < 0.05$) in methane (g/d) for sunflower and canola seed inclusions at 3.7% of diet DM as well as a reduction ($P < 0.05$) in methane g/kg DMI for the canola seed treatment relative to the control. The present study showed that methane (g/kg DMI) over the 127 days was lower ($P = 0.02$) for the corn oil cattle relative to the control. Guyader et al. (2015) fed linseed oil to cows and observed a reduction in methane production (g/kg DMI). The present study showed a reduction ($P < 0.01$) in methane (g/kg ADG) of 15% when corn oil was included compared to the control. This shows that the more efficient the animal is, the less methane produced per production unit even though factors such as DMI were reduced by supplementing corn oil.

When analyzing data as repeated measures, dry matter intake while in the methane chambers was not different between corn oil and control treatments ($P = 0.70$; Table 3.5). The difference between treatments for DMI was consistent, but with only 5 d instead of 127 d, the differences were not detected for DMI while in the chambers. Methane production (g/d) was greater as period progressed, regardless of treatment, shown by the period effect ($P < 0.01$; Figure 3.1). Methane production increasing as periods progressed could be a result of as the animal gets larger they tend to produce more methane, even though DMI stayed constant and did not follow this trend. There was not a treatment by period interaction for methane production (g/d; $P = 0.18$). Methane (g/kg DMI while in the chamber) was numerically lower, although not significant ($P = 0.29$) when corn oil was included compared to the control as it was reduced by 13%.

Carbon Dioxide

Carbon dioxide production (g/d) was not different (P = 0.38) between the corn oil and control treatments (Table 3.6). No other studies have reported the effect of corn oil on carbon dioxide production, but other oils have been examined. Guyader et al. (2015) fed a linseed oil to non-lactating cows and reported a tendency (P = 0.06) for carbon dioxide to be reduced compared to the control. Sauer et al. (1998) reported that adding soybean oil at 3.5% of diet DM to the diet of lactating cows did not affect carbon dioxide production (l/d) relative to the control. The current study showed no differences in carbon dioxide production (g/kg DMI) between treatments (P = 0.80). Guyader et al. (2015) showed similar results as well, with no differences in carbon dioxide per kg DMI when feeding linseed oil. In this experiment, carbon dioxide per kg ADG was not different between treatments (P = 0.19). The ratio of CH_4:CO_2 was not different between the corn oil and control treatment in this study (P = 0.17). When analyzed as repeated measures, carbon dioxide production (g/d) did not differ between treatments over the three sampling periods (P = 0.38; Table 3.6). Carbon dioxide per kg of DMI while in the methane barn during the sampling periods was not different between treatments (P = 0.67), there was not a sampling period effect (P = 0.43), and no interaction between period and treatment occurred (P = 0.55).

Manure

Methane and carbon dioxide were measured from the manure for one day following the 5 d collection period with cattle. The procedure is the same as described in Exp. 1. Methane production from manure was 0.87 g/steer daily (SD = 1.12) and the

carbon dioxide from manure was 249 g/steer daily (SD = 173). The results are variable due to difficulties in removing all of the manure the same way between each collection period. Baseline carbon dioxide levels were 933 g/steer daily when manure and cattle were removed from the chambers and is contributing to the carbon dioxide measurements observed with manure and cattle in the chamber. Baseline methane levels are 1.9 g/steer daily when manure and cattle are removed from the chamber, which contributes to the methane measurements reported with manure and cattle in the chamber. Emissions from manure were calculated by taking the levels recorded with manure in the chamber and subtracting the baseline levels recorded after manure removal from the chamber. Baseline emission levels are greater than what was reported in experiment 1, and could be a result of inconsistent manure removal between trials as well as between periods within trial. The majority of the pen surface is soil, so it is difficult to remove all manure excreted by cattle, or just manure without soil contamination. The amount of methane and carbon dioxide produced from manure appears to be negligible but may be underestimated with these methods.

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Table 3.1. Composition of diet (DM-basis) for Ad-libitum and Limit-Fed Cattle (Exp. 1)

Ingredient, % of diet DM	Ad-Libitum and Limit-Fed[1]
Alfalfa	45
Sorghum Silage	30
Modified distillers grains plus solubles	22
Supplement[2]	
Fine Ground Corn	2.547
Tallow	0.075
Salt	0.300
Beef Trace Mineral[3]	0.050
Vitamin A-D-E[4]	0.015
Rumensin[5]	0.013

[1]Limit-Fed = Restricted to 75% of Ad-libitum cattle DMI

[2]Supplement fed at 3% diet DM

[3]Premix contained 10% Mg, 6% Zn, 2.5% Mn, 0.5% Cu, 0.3% I, and 0.05% Co

[4]Premix contained 1,500 IU of vitamin A, 3,000 IU of vitamin D, and 3.7 IU of vitamin E per g

[5]Formulated to supply Rumensin-90 (Elanco Animal Health, Greenfield, IN) at 26 mg / kg

Table 3.2. Composition of diets for control vs. corn oil treatments (Exp. 2)

Ingredient, % of diet DM	Control	Corn Oil
Dry-rolled corn	33	31.5
High-moisture corn	33	31.5
Wet distillers grains plus solubles	15	15
Corn silage	15	15
Corn oil	-	3
Supplement[1]		
Fine ground corn	1.368	1.368
Limestone	1.640	1.640
Tallow	0.100	0.100
Urea	0.500	0.500
Salt	0.300	0.300
Beef Trace Mineral[2]	0.050	0.050
Vitamin A-D-E[3]	0.015	0.015
Rumensin[4]	0.017	0.017
Tylan[5]	0.011	0.011

[1]Supplement fed at 4% diet DM

[2]Premix contained 10% Mg, 6% Zn, 2.5% Mn, 0.5% Cu, 0.3% I, and 0.05% Co

[3]Premix contained 1,500 IU of vitamin A, 3,000 IU of vitamin D, and 3.7 IU of vitamin E per g

[4]Formulated to supply Rumensin-90 (Elanco Animal Health, Greenfield, IN) at 33 mg / kg

[5]Formulated to supply Tylan-40 (Elanco Animal Health) at 9.7 mg / kg

Table 3.3. Effects of ad-libitum vs. limit-feeding cattle on performance for growing diets (Exp. 1)

	Ad-Libitum	Limit-Fed[1]	SEM	*P*-value
Initial BW, kg	274	274	1	0.76
Ending BW, kg	380	354	2	< 0.01
DMI, kg / d[2]	8.4	6.2	0.1	< 0.01
DMI, kg / d[3]	8.4	6.5	0.2	< 0.01
ADG, kg	1.01	0.77	0.02	< 0.01
G:F	0.121	0.125	0.002	0.40

[1]Limit-Fed = Restricted to 75% of Ad-libitum cattle DMI

[2] DMI over the 105 d trial

[3]DMI while in the methane barn during period 3

Table 3.4. Effect of ad-libitum vs. limit-feeding cattle on methane and carbon dioxide production for growing diets (Exp. 1)

	Ad-Libitum	Limit-Fed[1]	SEM	*P*-value
Methane				
g / d	156	126	2	< 0.01
g / kg of DMI[2]	18.7	20.3	0.4	0.07
g / kg of DMI[3]	18.7	19.5	0.8	0.53
g / kg of ADG[4]	155	164	7	0.41
Carbon Dioxide				
g / d	6831	6032	163	0.04
g / kg of DMI[2]	818	974	22	0.02
g / kg of DMI[3]	816	937	45	0.16
g / kg of ADG[4]	6765	7856	346	0.11
$CH_4 : CO_2$	0.023	0.021	0.0003	0.02

[1]Limit-Fed = Restricted to 75% of Ad-libitum cattle DMI

[2]DMI over the 105 d trial

[3]DMI while in the methane barn during period 3

[4]ADG over the 105 d trial

Table 3.5. Effects of corn oil supplementation (3% of diet DM) in finishing diets on cattle performance and carcass characteristics (Exp. 2)

	Control	Corn Oil	SEM	*P*-value
Performance				
Initial BW, kg	370	369	1	0.72
Final BW, kg	591	596	4	0.39
DMI, kg / d[1]	11.7	11.2	0.1	0.02
ADG, kg / d	1.74	1.80	0.02	0.14
G:F	0.150	0.161	0.003	0.02
Carcass Characteristics				
HCW, kg	372	376	2	0.34
LM area, cm^2	82.0	84.2	1.1	0.27
Fat thickness, cm	1.44	1.39	0.07	0.60
Marbling score[2]	497	484	9	0.43
Calculated YG[3]	2.98	2.85	0.09	0.35

[1]DMI over the 127 d trial

[2]Marbling score: 400 = $Slight^{00}$, 450 = $Slight^{50}$, 500 = $Small^{00}$, etc.

[3]YG = 2.50 + (0.9843 * rib fat thickness, cm) + (0.2 * 2.5% KPH), + (0.0084 * HCW) – (0.0496 * LM area, cm^2) (USDA, 2016)

Table 3.6. Effects of corn oil supplementation (3% of diet DM) on methane and carbon dioxide production from cattle fed finishing diets (Exp. 2)

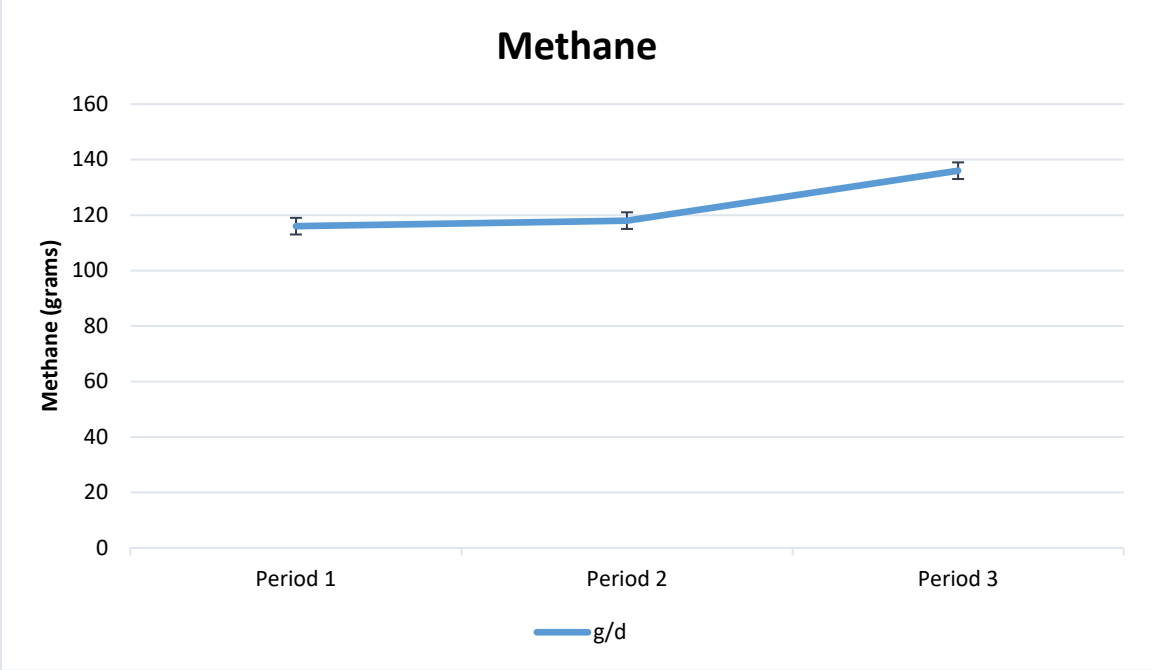
	Control	Corn Oil	SEM	*P*-value	
				TRT	Period
DMI, kg[2]	10.8	10.4	0.5	0.70	0.81
Methane					
g / d	132	115	3	0.03	< 0.01
g / kg DMI[1]	11.3	10.1	0.2	0.02	-
g / kg DMI[2]	12.8	11.1	0.9	0.29	0.56
g / ADG[3]	75.7	64.1	1.0	< 0.01	-
Carbon Dioxide					
g / d	10,907	10,538	252	0.38	0.31
g / kg DMI[1]	938	926	31	0.80	-
g / kg DMI[2]	1072	1016	83	0.67	0.43
g / ADG[3]	6,280	5,873	170	0.19	-
$CH_4 : CO_2$	0.012	0.011	0.001	0.17	0.08

[1]DMI over the 127 d trial

[2]DMI in the methane barn across all 3 periods of collection

[3]ADG over the 127 d trial

Figure 3.1. Effect of period on methane production (g/day and g/kg of DMI) (Exp. 2)



Description: Effect of period on methane production, treatment averages combined. There was an effect of period ($P < 0.01$) for methane production (g/d), with greater levels of methane being produced as periods progressed. Standard error = 3 for methane g/d

CHAPTER IV. Evaluation of the Effects of Biochar on Methane Production In Vitro, and Digestibility and Methane Production In Vivo

T.M. Winders, M.L. Jolly-Breithaupt, H.C. Wilson, P.J. Kononoff, G.E. Erickson, A.K. Watson

Department of Animal Science, University of Nebraska, Lincoln, NE 68583-0908

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[2]Corresponding author: C220 Animal Science Building, Phone: 402-472-6058; Fax: 402-472-6362; email: awatson3@unl.edu

Abstract

An in vitro study and an in vivo study evaluated the effects of biochar on methane production from cattle. For the in vitro study, a 3×4 factorial design was used to test three types of biochar (pine, bone, cedar) at four inclusion (0, 3, 6, and 9% of DM added to in vitro tubes). A fourth biochar treatment (raw) was analyzed separately with different inclusions (0, 0.8, 3, and 6%). As inclusion increased, total gas (mL) and methane production (mL) increased linearly ($P \leq 0.07$). A treatment difference ($P < 0.01$) was observed between pine, cedar, and bone treatments for both total gas and methane production (mL). After the in vitro trial, a digestion trial examined the effects of biochar inclusion (0, 0.8, and 3% of diet DM) on diet digestibility and methane production from cattle. A linear decrease in NDF digestibility ($P = 0.08$) was observed as biochar inclusion increased, while quadratic effects were observed for DE intake (DEI; $P = 0.08$) and OMD ($P = 0.10$), with 0.8% inclusion being the greatest. Linear tendencies were observed for ADF intake ($P = 0.13$) and NDF excreted ($P = 0.14$) with 3% being the greatest, while a quadratic tendency was observed for DMD ($P = 0.11$) with 0.8% being the greatest. Methane production (g/kg of DMI, g/Mcal of GE intake) was not reduced by increasing inclusion of biochar ($P \geq 0.17$), however, a quadratic effect ($P = 0.05$) was observed for methane production as g/Mcal of DEI with 0.8% being lower than 0 and 3%. Methane production (g/d) tended to derease quadratically with 0.8% inclusion having the lowest emissions. Methane (g/Mcal of DEI) was reduced ($P = 0.07$) and methane production measured as g/d, g/kg of DMI, and g/Mcal of GEI tended to be reduced ($P \leq 0.13$) when analyzed as biochar inclusion vs. no biochar inclusion. Carbon

dioxide production was also reduced by biochar when analyzed as increasing inclusion or as biochar versus no biochar on a g/d and g/kg of DMI basis ($P \leq 0.06$).

Key words: biochar, calorimetry, cattle, methane

Introduction

Energy lost as methane by ruminants can range from 2-12% of total gross energy intake (GEI), but is variable depending on diet composition and energy density. Diets lower in digestibility (growing diets) tend to produce the upper range of methane lost as a % of GEI (12%), whereas highly digestible diets (finishing diets) tend to produce methane at only 2-3% of GEI (Johnson and Johnson, 1995). However, total methane amounts (g/d) can be greater for finishing diets relative to growing diets due to a larger DMI and typically larger cattle consuming these diets. Even though total daily methane production can be greater for finishing cattle, a more energy dense diet leads to faster animal growth and therefore less days until harvest per animal, resulting in less methane per kg of meat (Boadi et al., 2004).

Increasing diet energy is an effective methane mitigation strategy and there are other strategies being tested. One such strategy is through dietary manipulation of the rumen environment. Methanogens produce methane in the rumen, so some methanogen-inhibiting feed additives have been examined. A commonly used rumen modifier is the ionophore monensin, which alters the microbial population resulting in increased propionate production. Monensin has been reported to decrease methane by up to 25% (Johnson and Johnson, 1995) but results have been variable on the duration of methane suppression (Guan et al., 2006).

Biochar is another feed product with potential as a methane inhibitor. Biochar is produced by burning OM (typically plant material) at very high temperatures in the absence of oxygen (Hansen et al., 2012). Although biochars' mode of action is not fully

understood, it has been suggested that it adsorbs gas in the rumen resulting in reduced methane eructation. Leng et al. (2012a) proposed several other theories. One is that the porous nature of biochar will increase the amount of inert surface area in the rumen, allowing for improved habitat for microbes to reside. This may increase microbial growth, allow feeds to be digested more completely, and bring methanogens and methanotrophs together, leading to more complete oxidation of feeds and less methane production. Feeding biochar has resulted in methane reduction in vitro (Hansen et al., 2012; Leng et al., 2012a) and in vivo (Leng et al., 2012b).

The objectives of the following experiments were to determine if adding biochar to an in vitro system reduces methane production and if the feedstock used to make the biochar affects this response. Furthermore, the effects of biochar on methane production and diet digestibility were evaluated in vivo.

Materials and Methods

All animal care and management practices were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee.

Experiment 1: In vitro

A 3×4 factorial design was used to determine the effects 3 types of biochar, included at 4 inclusions, have on total gas production and methane production in an in vitro system. Treatments were due to the feedstock used to make the biochar: pine tree, cedar tree, and cattle bone. Four inclusions of biochar were tested, 0, 3, 6, and 9% on a DM basis (Table 4.1). A fourth biochar was also examined (mixed feedstock), at inclusions of 0, 0.8, 3, and 6% of diet DM. The mixed feedstock biochar was derived

from a blend of wood-based substrates. The mixed feedstock biochar was fed in the in vivo study and the in vitro inclusions were selected in order to test inclusions similar to those used in the in vivo study. The raw treatment was analyzed in two separate in vitro runs (replications) while the other treatments were analyzed in 1 run.

Biochar was ground to 1-mm added to in vitro bottles on top of a ground base diet of 45% alfalfa, 30% sorghum silage, and 25% modified distillers grains plus solubles on a DM basis (Table 4.1). The diet was composited into a whirl pack bag as a complete diet and then weighed into 250-mL gas production bottles (Ankom Technology, Macedon, NY). The diet was added at 1.000 – 1.040 g per bottle. The biochar was then added to the bottles on top of the 1g of diet. Two bottles per run were left blank (no feed or biochar), in order to quantify gas production from the rumen inoculum. The diet and biochar used in the in vitro bottles were ground to 1-mm particle size.

Inoculum was obtained from two fistulated steers consuming a high-roughage diet (Table 4.1). Approximately 1.5 L of rumen fluid was collected from each steer by taking rumen contents and squeezing the fluid into a 4-layer cheesecloth filter prior to being stored in a pre-warmed thermos (Thermos LLC, Schaumburg, IL) container, for a total of 3 L of rumen fluid collected for each in vitro run. The fluid was then poured into three 1-L separatory funnels, flushed with an inlet of carbon dioxide, and closed with a rubber stopper. The funnels were placed in a 39°C water bath until the particles in the fluid formed a layer on top of the liquid. The liquid portion was drained and mixed with reduced McDougall's Buffer which contained 1.5 g urea/L, while the particles stayed in the funnel (Weiss, 1994). McDougall's Buffer was mixed at a concentration of 176.4

g/18L potassium chloride (KCl), 8.46 g/18L sodium chloride (NaCl), 2.16 g/18 L magnesium sulfate ($MgSO_47H_2O$) and 2.9 g/18 L calcium chloride ($CaCl_22H_2O$) to create artificial saliva. This mixture was raised to 39°C in the water bath prior to being mixed with the filtered and separated rumen fluid.

One hundred mL of rumen fluid - McDougall's Buffer mixture (50:50 blend) was added to twenty 250-mL in vitro gas bottles per in vitro run. Each bottle was flushed with carbon dioxide prior to being sealed with a gas production module that fastened to the top of the 250 mL bottles (Ankom Technology). After all gas bottles were filled and sealed, they were lightly swirled to mix the diet into the inoculum. The bottles were then placed into a 39°C water bath for 24 hours, and the bottles were swirled twice a day. The water bath kept the in vitro bottles at the same temperature experienced in the rumen. Once all bottles were placed in the water bath, batteries were placed in the module and communication with the Ankom system began. An ambient module was set outside of the water bath and was used to measure only ambient pressure levels, while all other modules measured the pressure inside the in vitro bottles. Once gas pressure reached 2 kPa, it was automatically released from the bottle to stop greater levels of pressure from accumulating. Gas production occurred continuously for 24 hours, while the pressure was collected every 5 minutes over the 24 hours and then converted to gas production values, as described later.

Methane was measured once at the end of the 24-hour period by taking 5 mL of gas out of the septum port attached to the bottle using a syringe (Hamilton Co, Reno, Nevada). The gas sample was then injected into an SRI 8610C Gas Chromatograph (GC)

(SRI Instruments, Torrance, CA) to be analyzed for concentration of methane and carbon dioxide over a two-minute cycle in the GC. This process occurred for each bottle, including the two blanks that were included in every run.

Gas Calculations

To calculate total gas production, the pressure measurements collected during the 24-hr run were converted using the ideal gas law ($n = p(V/RT)$) and Avogadro's law. Using the ideal gas law, n = moles (mol) of gas produced, p = pressure in kilopascal (kPa), v= headspace volume (liters) in the in vitro bottles, T = temperature (K) and R = the gas constant (8.314472 L × kPa × K^{-1} × mol^{-1}). Avogadro's law states that at atmospheric pressure in psi (1 psi = 6.894757293 kPa), 1 mol accounts for 22.4 L at 273.15°K and 101.325 kPa. A mol of gas can be converted to mL by using this equation: gas produced (mL) = n × 22.4 × 1000. These calculations to obtain total gas production were done using the pressures from each in vitro bottle obtained at the end of the 24-hr run. The pressure recorded by the Ankom system (Ankom Technology) was in psi and was converted to the equivalent kPa at 39°C by multiplying by 6.894757293 to obtain the needed pressure unit (kPa) for the ideal gas law.

Total volume in the in vitro bottle (310 mL) minus the occupied space (100 mL) yields the headspace volume (V) used in the ideal gas law, equaling 210 mL, or 0.21 L. The gas constant (R) used is the previously stated 8.314472 L × kPa × K^{-1} x mol^{-1} value. Temperature (T) used in this equation was calculated by summing 273°K + 39°C to equal 312°K. When calculating gas production for each bottle across each run, the same V, R, and T values were used, leaving p to be variable depending on the individual in vitro

bottle pressure. The p value was then multiplied by 0.000080952 L/L × kPa × K^{-1} × mol^{-1}, which is the product of (V/RT), × K to calculate the n value used in the previously stated conversion that results in gas produced (mL). The values obtained from the in vitro bottles that only contained rumen fluid and McDougall's Buffer were subtracted from each treatment-containing in vitro bottle gas production value to correct for gas production from the rumen fluid. These corrected values were then used to calculate methane and total gas production using the following equations:

Total gas produced (mL) = pressure (psi) × 6.894757293 kPa × 0.000080952 L/ L·kPa·K^{-1}·mol^{-1} × K × 22.4 L/mol × 1000 ml/L

Methane produced (mL) = CH_4 concentration (mg/kg)/1000000 × gas volume (mL)

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., 2013, Cary, NC). The first three biochar types were analyzed as a 3×4 factorial with treatment (biochar type), inclusion, and treatment by inclusion interaction included in the model as fixed effects. For the in vitro data using mixed feedstock biochar run was included in the model as a random effect and inclusion was a fixed effect. Each inclusion within treatment was replicated 5 times, however, some Ankom modules failed to release pressure, resulting in those in vitro bottles being removed from the analysis. Final data analyzed for bone biochar included 5, 5, 3, and 5 bottles for the 0, 3, 6, and 9% inclusion, respectively. For Cedar biochar this was 3, 5, 4, and 4 bottles and for Pine biochar 2, 4,

3, and 2 bottles were included. Total bottles for the mixed feedstock biochar included 9, 10, 8, and 10 bottles for 0, 0.8, 3, and 6% inclusion, respectively. . Probabilities were considered significant at $P < 0.05$ and tendencies are discussed at $P \leq 0.10$.

Experiment 2

An indirect calorimetry study evaluated methane production by growing cattle with varying inclusions of Biochar (High Plains Biochar LLC, Laramie, WY). Biochar was analyzed for dioxin and furan contaminants (Pace Analytical, Minneapolis, MN). The presence of polychlorodibenzo-p-dioxins and polychlorodibenzofurans (US EPA Method 1613B) were non-detectable with a detection range of 1-10 ng/kg. Six crossbred steers (initial BW 529 kg; SD = 16 kg) were used in a 6 period crossover design. Steers were blocked by BW and assigned randomly within block to one of three treatments. Periods ranged from 14-24 days with two consecutive, 23-h periods in the headbox calorimeter, availability of the calorimeters dictated period length. Periods 1, 2, 5, and 6 were 14 d and periods 3 and 4 were 24 and 21d, respectively. Diets fed were identical between treatments other than inclusion of biochar included, which displaced fine ground corn in the supplement (Table 4.2). Corn silage was included at 30%, brome hay at 21%, wheat straw at 20%, wet distillers grains plus solubles (WDGS) at 22% and the supplement at 7% of the diet on a DM basis. Biochar was included at 0, 0.8 and 3% of the total diet and was mixed in the supplement. Urea was included in all diets at 0.5% of diet DM and treatments provided 200 mg/animal daily of monensin (Rumensin, Elanco Animal Health, Greenfield, IN).

Diets were mixed approximately twice a week and fed *ad libitum* twice daily at 0800 and 1500. Feed refusals were weighed back daily and adjustments for feed offered were made accordingly. Feed refusals were weighed, subsampled, and dried at 60°C for DM determination during the fecal collection period. Each period consisted of: adaptation to the treatments (minimum of 8 d), fecal grab sampling 4 times/d on 4 days leading up to headbox collections, and headbox collections for the final 2 d of the period. Fecal samples were composited by d, freeze dried, and ground to 1-mm using a Wiley Mill (Thomas Scientific, Swedesboro, NJ). The ground samples were then composited by period for each steer. Feed and fecal samples, composited by period, were dried at 100°C for 24 hours to determine DM and then burned in a cool muffle furnace at 600°C for 6 h to determine OM. Feed and fecal samples were analyzed for NDF using the Van Soest et al. (1991) method. Alpha amylase was added at the beginning of boiling and at 30 min of reflux in 0.5 mL increments to all fecal, corn silage, WDGS, and supplement samples. Sodium sulfite (0.5 g) was added to the samples before 100 mL of NDF solution was added. Feed and fecal samples were analyzed for ADF as well using method 973.18 (AOAC International 2000), with the addition of sodium sulfite (0.5 g). Acid insoluble ash was determined by placing the dried ADF sample into a cool muffle furnace at 600°C for 6 h. This was done to estimate fecal output and digestibility of the diet based off of this internal marker found in the feed and feces. Fecal output was calculated by dividing acid insoluble ash DMI by acid insoluble ash found in the feces. Digestibility can then be determined using the following equation: (DMI – fecal output)/DMI. Acid insoluble ash analysis was done on the base diet fed, feed refusals, and fecals to determine acid insoluble ash intake and fecal output, which was used to

determine the DM digestibility (DMD) of the three treatments. Gross heat energy was determined for feed and fecal samples using a Parr 6400 oxygen bomb calorimeter (Parr Instrument Company, Moline, IL). Digestible energy was then calculated by subtracting total gross fecal energy from total gross energy intake.

Gas Emissions

Methane emissions were measured through indirect calorimetry using headboxes built at the University of Nebraska-Lincoln. Three headboxes were available, so the light block started two days before the heavy block. The collections consisted of two consecutive, 23-h periods on the final two days of each period. The collection method was similar to that described by Foth et al. (2015). A training period was done prior to the experiment to get the steers acclimated to the headboxes. One steer was removed from the trial after period two because of a lack of DMI while in the headbox. Feed was offered *ad libitum* while the steers were in the headboxes and was adjusted based off refusals throughout the collection period. Feed was placed in the headbox when the steers entered at 0800. The doors were then closed and the vacuum motor (Model 115923, Ametek Lamb Electric, Kent, OH) was turned on, creating a negative pressure system in the headbox. Total airflow through the headbox was measured using a gas meter (Model AL425, American Meter, Horsham, PA), and was regulated by flow meters (Model 1350E Sho-Rate 50, Brooks Instruments, Hatfield, PA) to allow for proportional samples to be gathered. The headbox doors were closed 15 minutes prior to collection starting to allow for several air turnovers before emissions were collected. The samples were collected in foil bags that continuously and evenly filled throughout the 23h

collection period. Two bags per headbox were continuously filled over the 23h collection, one bag for ambient air entering the headbox and one for emissions leaving the headbox. Air was diverted to each bag using glass tube rotameters (Model 1350E Sho-Rate "50", Brooks Instruments, Hatfield, PA). These bags were analyzed for methane and carbon dioxide using a gas chromatograph (Universal Analyzers Inc, Carson City, NV).

Temperature and relative humidity was measured inside each headbox every minute using a probe (Model TRH-100, Pace Scientific Inc., Mooresville, NC) and collected using a data logger (Model XR440, Pace Scientific). These values are used in energy production calculations but were not utilized for this trial. After the 23h collection period, steers were brought back to their pens to rest for an hour while feed refusals were collected, rubber mats and waterers were cleaned, foil bags switched out, and flow rates were recorded. Gas measurements collected over the 2 d were averaged to obtain one value per period for each steer. A 5 d DMI average leading up to the 2 d headbox period was used to report gas emissions on a grams per kg of DMI basis. Feed refusals were accounted for by taking the average DM of the fed refusals during the fecal collection period and applying it to the average 5 d DM offered leading up to the headbox period. At the conclusion of the trial, the cattle were euthanized under veterinary supervison and composted because biochar is not an approved feed additive.

Statistical Analysis

Statistical analysis was done using the MIXED procedure of SAS for DM digestibility as a 6×6 balanced replicated Latin square and gas production as an

unbalanced replicated Latin rectangle (due to removal of one steer). The model included treatment, period, and block as fixed effects for digestibility analysis. Gas production analysis included treatment and period in the model. Steer was considered a random effect in both analyses. Gas production was analyzed using the differing inclusions of biochar (linear and quadratic contrasts) as well as biochar included versus biochar absent from the diet (i.e. combining the 0.8 and 3% treatments). Treatment by period interactions were tested for digestibility and gas production. Because treatments were not evenly spaced, the IML procedure of SAS was used to generate coefficients used for contrast statements. Probabilities were considered significant at $P < 0.10$ and tendencies are discussed at $P \leq 0.15$.

Results and Discussion

Experiment 1

Total gas production (mL) from the three types of biochar had a treatment ($P < 0.01$) and inclusion ($P = 0.04$) response (Table 4.3 and 4.4). The bone treatment produced the least gas, pine was intermediate, and cedar produced the most gas. As inclusion increased, a linear increase ($P < 0.01$) in total gas production (mL) was observed. Methane production (mL) also tended to linearly increase with increasing biochar inclusion ($P = 0.07$). Methane production as a percent of total gas production was greatest with no biochar (0.437%) and least for 3% biochar inclusion (0.419%). Type of biochar affected methane production (mL; $P < 0.01$), with the cedar treatment producing the least, bone being intermediate, and pine producing the most methane. As a percent of

total gas production, cedar biochar produced the least methane (0.404%) with pine being intermediate and bone being the greatest (0.476%). When analyzing the mixed feedstock biochar treatment (Table 4.5), increasing inclusion of biochar linearly increased ($P < 0.01$) total gas production. There was a quadratic increase in methane production ($P = 0.05$) as biochar inclusion increased. Methane production relative to total gas production averaged 0.416% and was consistent across treatments.

These results were not expected based off the literature, although data on biochar products are limited. Hansen et al. (2012) examined the effects of three types of biochar (gasified, wood-based, straw-based) on methane production in vitro. These authors used 0.5g feed samples that were sealed in filter bags and incubated in rumen fluid for 48 hours at 39°C. Biochar was added to the Ankom in vitro bottle outside of the sealed filter bag, and both were submersed in rumen fluid. Biochar was included at 9% of the diet DM. The authors found that total gas produced (g/diet g) was numerically lowered by 3-11% by adding the different biochar types; however, the reduction was not statistically significant. Methane production (CH_4 g/diet g) was numerically lowered by 11-17% for the biochar treatments relative to the control, but once again was not statistically reduced.

Leng et al. (2012a) conducted three in vitro experiments evaluating the effects of biochar on methane production. The biochar came from burning rice husks in a gasifier stove. In experiment 1, biochar was added at 5% of the diet DM and resulted in a reduction ($P \leq 0.03$) in methane as a percent of total gas, volume of methane produced (mL), and methane mL per g diet, compared to the control without biochar during the first 24 hours. Across hours 0-48 the same trend continued, with biochar reducing ($P \leq$

0.02) methane when analyzed in the same three ways. Experiment 2 from these authors evaluated the effects of inclusion of biochar, increasing from 1-5% of diet DM. All treatments reduced ($P \leq 0.01$) methane as a percent of total gas, mL of methane, and mL of methane per g of diet, however increasing the inclusion did not have an effect, as 1% reduced methane just as much as the 5% biochar inclusion. The authors speculated that even less biochar may be needed. In experiment 3, these authors included biochar at 0.5% and 1% of the diet DM and observed a reduction ($P < 0.01$) in methane (mL) of 10 and 12.7% respectively. A separate treatment included biochar (1% diet DM) plus nitrate (6% diet DM) and observed a reduction ($P < 0.01$) in methane (mL) of 49%.

Overall, Leng et al. (2012a) observed a 10-14% reduction in methane across the three in vitro experiments and Hansen et al. (2012) showed a numerical reduction of 11-17% for methane production (g/g of diet). These results differ from what was observed in this trial. One difference between trials is the substrate used to derive the biochar which ranged from cattle bone in this experiment to rice husks and straw-based biochar in the other experiments cited. Another difference is the diet that biochar was inoculated with as well as the inoculum used, which could lead to fermentation differences that could alter methane production. Leng et al. (2012a) used a diet consisting of 82% cassava root meal and 18% cassava leaf meal. Cassava root is high in soluble carbohydrates and low in fiber (Oguntimein, 1988). Hansen et al. (2012) used a diet that was 46.5% NDF and 8.6% starch. The diet used in the current trial is similar in NDF content to the Hansen et al. (2012) study but did not contain any starch, and included MDGS. Total digestible nutrients (TDN) for the current trail would have been lower than

Hansen et al. (2012) and Leng et al. (2012a) because no starch was present and MDGS, although shown to improve cattle performance, have lower ruminally availale energy than starch which could account for some of the differences observed between trials. The 24-hr time used in the present trial may not have been enough time for the microbes in the inoculum to adapt to the biochar. According to theories previously stated by Leng et al. (2012a), biochar could be acting as a habitat for methanotrophs to reproduce and to oxidize methane, but it may take longer than 24 hours for this process to start having an effect on methane production. In the present trial each in vitro run used different rumen fluid inoculum, and although the fluid was separated from the particles, small particles may still be present, leading to more or less fermentation from the inoculum. The microbial population could also vary between runs, even though it was obtained from cattle on a high roughage diet.

Experiment 2

Digestibility and Energy

Dry matter intake (kg/d) did not differ between treatments ($P \geq 0.43$; Table 4.6), but did increase between periods as a result of the cattle growing, and therefore eating more. This is similar to results reported by Leng et al. (2012b) in which authors fed biochar derived from rice husk to 12 local "Yellow" cattle in Laos. These authors conducted a 98d trial feeding biochar at 0.6% of the diet DM in a cassava root chip/cassava foliage based diet. No differences in DMI were detected, and the authors observed an increase in ADG and feed efficiency but did not report any digestibility measures of the diets fed.

All intake, fecal output and digestibility data are reported in Table 4.6. There were no differences between treatments except for OM digestibility (OMD) and NDF digestibility (NDFD). A quadratic increase ($P = 0.10$) was observed for OMD with the 0.8% biochar treatment having greater digestibility than the 3% treatment, with 0% treatment being intermediate and similar to both. Similarly, DM digestibility tended ($P = 0.11$) to increase quadratically. A linear ($P = 0.08$) decrease was observed for NDFD with 3% inclusion of biochar having the lowest digestibility. Gross energy intake (GEI; Mcal/d) and digestible energy intake (DEI; Mcal/d) did not differ between treatments ($P \geq 0.27$), however, DEI as Mcal/kg of DMI showed a quadratic response ($P = 0.08$) with 0.8% biochar being greater than 3%, while 0% treatment was intermediate and similar to both. A tendency was observed for a linear increase in NDF excretion ($P = 0.14$) and ADF intake ($P = 0.13$), while energy excreted (Mcal/d) tended to decrease quadratically ($P = 0.13$).

Van et al. (2006) fed a charcoal product derived from bamboo to goats on an acacia foliage and para grass based diet in Vietnam at inclusions of 0, 1, and 1.5 g per kg of BW. These authors reported that bamboo charcoal did not affect DMI, and improved DMD and OMD values for the 0.5 and 1 g/kg BW treatments compared to the control and 1.5% treatment. The authors attributed the digestibility improvements to the ability of the charcoal to adsorb toxins and tannins, preventing them from reaching the intestines and inhibiting enzyme excretion, resulting in more digestion. However, Kutlu et al. (2001) reported that wood-based biochar products are capable of adsorbing vitamins, fats

and enzymes when included at a high level, which could explain some of the digestibility responses observed in the present trial for the 3% biochar treatment.

Methane and Carbon Dioxide Production

Three inclusions

Reported DMI used for gas emission calculations was a 5 d average prior to cattle entering the headboxes, and was not different between treatments ($P \geq 0.68$; Table 4.7). Methane production (g/d) was not different between treatments; however, a tendency for a quadratic effect ($P = 0.14$) was observed with the 0.8% biochar treatment reducing methane compared to the 0% treatment while 3% was intermediate. Numerically, the 0.8% biochar treatment reduced methane (g/d) by 11% compared to the control treatment without biochar. This is a smaller response than Leng et al. (2012b) reported with a 24% reduction in methane (ppm) when feeding biochar derived from rice hulls at 0.6% of the diet DM. Methane production (g/kg DMI) was not different between treatments in the present study, and GEI and DEI based off the 5 d intakes were also not different ($P \geq 0.18$). When analyzing methane produced per Mcal of GEI no differences were observed between treatments ($P \geq 0.17$); however, methane per Mcal of DEI was lowest for 0.8% biochar and greatest for the 0% treatment, resulting in a quadratic response ($P = 0.05$) with 3% biochar being intermediate and similar to both.

Carbon dioxide production (g/d) was affected by treatment with 0% biochar having the greatest carbon dioxide production and 0.8% biochar reducing carbon dioxide the most, resulting in a quadratic decrease ($P = 0.05$). This trend continued for carbon

dioxide per kg of DMI with 0.8% reducing carbon dioxide the most creating a quadratic response ($P = 0.06$). Leng et al. (2012b) reported greater carbon dioxide production from the biochar treatment relative to the control, which differs from the present trial, but did not suggest why this may have occurred. These same authors reported a lower ($P < 0.01$) CO_2:CH_4 ratio for the biochar-fed cattle, however in the present study the ratio was not affected by treatment ($P \geq 0.67$).

Biochar vs. No Biochar

When combining the two treatments that contained biochar (0.8 and 3%) into one to compare to the 0% treatment, DMI was not different ($P = 0.70$; Table 4.7). Methane production (g/d) tended ($P = 0.11$) to be lower for the biochar cattle relative to the control cattle. Methane (g/kg DMI; $P = 0.13$) and per Mcal of GEI ($P = 0.11$) also tended to be reduced for the biochar treatment compared to the control while methane per Mcal of DEI was reduced ($P = 0.07$) for the biochar cattle. Carbon dioxide production was also reduced ($P \leq 0.03$) with the inclusion of biochar when reported as g/d and g/kg of DMI compared to the control.

The reduction in methane production reported by Leng et al. (2012b) was not observed to the same extent in the present study. Those authors reported a 24% reduction in methane when feeding biochar at 0.6% of the diet. In the current trial, with all 3 treatments analyzed, methane production was not statistically reduced. However, methane reported as g/d and g/kg tended to be 9.4% and 8.8% lower, respectively, when analyzed as two treatments, with and without biochar in the diet. Leng et al. (2012b)

also observed a 13% reduction in carbon dioxide (ppm) when including biochar in the diet. Carbon dioxide production was reduced approximately 8% in the current trial.

There could be many reasons for the different magnitude of results observed between the present trial and what Leng et al. (2012b) reported, including cattle breed, cattle size, diet consumed, and collection method. These authors reported that the 12 "Yellow" cattle they used had an initial BW of 80-100 kg, whereas in the present trial the cattle used were roughly 5 times that size. Rumen function and microbial population within the rumen certainly vary between cattle that are of different breed, size, and consumption, which could influence the results reported. In the Leng et al. (2012b) study authors used a short-term collection method for measuring respired air (once for 5 minutes in a headbox) and calculating methane production as described by Madsen et al. (2010). As illustrated in chapter three, intake drives methane production, so short-term measurements are variable depending on time of gas collection relative to feeding.

Conclusion

The effect of biochar on methane production in vitro and in vivo has not been explored in depth, but has shown some promise as a potential mitigation strategy. Hansen et al. (2012) and Leng et al. (2012a) both reported reduced methane emissions from biochar of 10-17% when examining the effects in vitro, although Hansen et al. (2012) did not report statistically significant differences. The in vitro runs in the present

trial showed an increase in methane production rather than a reduction and a concurrent increase in total gas production. It is possible that using inoculum from cattle consuming biochar would have resulted in reduced methane production in vitro. In vitro runs are variable and do not replicate what happens inside the animal perfectly as there are sources of error involved in the procedure. For this reason, the in vivo trial was conducted. There was a tendency for a reduction in methane production when the data were analyzed as biochar inclusion vs. no biochar. The effects of biochar on the microbial population, and subsequent methane production are not fully understood, but the theories reported Leng et al. (2012a) show promise. There is a need for more research to gain a better understanding of the mode of action.

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Table 4.1. Composition of diet (DM-basis) fed to fistulated donor steers and diet incubated in vitro (Exp. 1)

Ingredient, % of diet DM	Fistulated Steers	In vitro Diet
Brome hay	70.5	-
Dry rolled corn	5.84	-
Dry distillers grains plus solubles	23.3	-
Supplement		
Beef Trace Mineral[1]	0.05	-
Vitamin A-D-E[2]	0.03	-
Salt	0.28	-
Alfalfa	-	45
Sorghum Silage	-	30
Modified distillers grains plus solubles	-	25

[1]Premix contained 10% Mg, 6% Zn, 2.5% Mn, 0.5% Cu, 0.3% I, and 0.05% Co

[2]Premix contained 1,500 IU of vitamin A, 3,000 IU of vitamin D, and 3.7 IU of vitamin E per g

[3]Inclusion for pine, cedar, and bone biochar were 0, 3, 6, and 9% of diet DM. Added in addition to the 1g diet sample

[4]Inclusion used for mixed feedstock biochar were 0, 0.8, 3, and 6% of diet DM. Added in addition to the 1g diet sample

Table 4.2. Composition of diet (DM-basis) fed to cattle (Exp. 2)

	Biochar, % Inclusion		
Ingredient, % of diet DM	0	0.8	3
Brome hay	21	21	21
Wheat straw	20	20	20
Corn silage	30	30	30
Wet distillers grains plus solubles	22	22	22
Supplement[1]			
Fine ground corn	4.630	3.830	1.630
Biochar	-	0.800	3.000
Limestone	1.320	1.320	1.320
Tallow	0.175	0.175	0.175
Urea	0.500	0.500	0.500
Salt	0.300	0.300	0.300
Beef Trace Mineral[2]	0.050	0.050	0.050
Vitamin A-D-E[3]	0.015	0.015	0.015
Rumensin-90[4]	0.010	0.010	0.010

[1]Supplement fed at 7% diet DM

[2]Premix contained 10% Mg, 6% Zn, 2.5% Mn, 0.5% Cu, 0.3% I, and 0.05% Co

[3]Premix contained 1,500 IU of vitamin A, 3,000 IU of vitamin D, and 3.7 IU of vitamin E per g

[4]Formulated to supply Rumensin-90 (Elanco Animal Health; Greenfield, IN) at 20 mg / kg

Table 4.3. Total gas and methane production measured in vitro at 24 hours for biochar made from 3 different substrates (Exp. 1)[1]

	TRT				
	Bone	Pine	Cedar	SEM	*P*-value
Total Gas Production, mL	91.9[a]	112[b]	100[c]	2.0	< 0.01
Methane production, mL	0.437[a]	0.462[b]	0.404[c]	0.009	< 0.01
Methane as a % of total gas, mL	0.0047[a]	0.0041[b]	0.0040[b]	0.00005	< 0.01

[1]Biochar was added at 4 inclusions (0, 3, 6, and 9% of diet DM). The interaction between inclusion and biochar type was not significant ($P \geq 0.11$) and main effects of biochar type are shown.

[a, b, c] Means within a row with different superscripts are different ($P < 0.05$)

Table 4.4. Total gas and methane production measured in vitro at 24 hours for biochar at differing inclusions (Exp. 1)[1]

	Inclusion					*P-value*	
	0	3	6	9	SEM	Lin[1]	Quad[2]
Total Gas Production, mL	96.5[a]	101[ab]	105[b]	104[b]	2.2	< 0.01	0.25
Methane production, mL	0.422	0.423	0.447	0.445	0.016	0.07	0.88
Methane as a % of total gas, mL	0.0044	0.0042	0.0043	0.0043	0.00005	0.34	0.11

[1]Biochar was made from 3 different substrates (bone, pine, cedar). The interaction between inclusion and biochar type was not significant ($P \geq 0.11$) and main effects of inclusion are shown.

[a, b] Means within a row with different superscripts are different ($P < 0.05$)

Table 4.5. Total gas and methane production measured in vitro at 24 hours for differing inclusions of the mixed feedstock biochar (Exp. 1)

	Inclusion					*P-value*	
	0	0.8	3	6	SEM	Lin[1]	Quad[2]
Total Gas Production, mL	84.4[a]	87.8[a]	93.4[b]	94.0[b]	3.4	< 0.01	0.08
Methane production, mL	0.351[a]	0.365[a]	0.389[b]	0.390[b]	0.012	< 0.01	0.05
Methane as a % of total gas, mL	0.0039	0.0039	0.0040	0.0040	0.0002	0.07	0.40

[a, b] Means within a row with different superscripts are different ($P < 0.05$)

Table 4.6. Effect of biochar inclusion on intake and total tract digestibility (Exp. 2)

	Biochar Inclusion, % DM				*P-value*	
	0	0.8	3	SEM	Lin[1]	Quad[2]
DM						
Intake, kg/d	8.01	7.88	7.83	0.21	0.43	0.64
Excreted, kg/d	3.57	3.35	3.57	0.16	0.71	0.18
Digestibility, %	55.7	57.6	54.7	1.12	0.25	0.11
OM						
Intake, kg/d	7.25	7.16	7.12	0.19	0.52	0.74
Excreted, kg/d	3.02	2.83	3.03	0.14	0.68	0.18
Digestibility, %	58.6[ab]	60.6[a]	57.7[b]	1.16	0.31	0.10
NDF						
Intake, kg/d	4.24	4.19	4.28	0.11	0.62	0.57
Excreted, kg/d	2.11	2.00	2.24	0.11	0.14	0.16
Digestibility, %	50.5[ab]	52.6[a]	48.2[b]	1.55	0.08	0.10
ADF						
Intake, kg/d	2.83	2.82	2.93	0.08	0.13	0.53
Excreted, kg/d	1.52	1.47	1.63	0.08	0.16	0.33
Digestibility, %	46.7	48.1	45.0	1.50	0.29	0.35
Energy						
GEI, Mcal/d	35.3	34.8	34.8	0.93	0.62	0.68
Fecal Energy, Mcal/d	14.8	13.8	14.8	0.68	0.67	0.13
DEI, Mcal/d	20.5	21.0	20.0	0.51	0.27	0.30
DEI, Mcal/kg DMI	2.57[ab]	2.68[a]	2.56[b]	0.05	0.52	0.08

[1]Linear effect on response variables

[2]Quadratic effect on response variables

[a, b,] Means within a row with different superscripts are different ($P < 0.10$)

Table 4.7. Effect of increasing inclusion of biochar on methane and carbon dioxide emissions from steers (Exp. 2)

	Biochar Inclusion, % DM				*3 Types P-value*		*Bio vs No Bio*[3]
	0	0.8	3	SEM	Lin[1]	Quad[2]	*P*-value
DMI, kg	7.9	7.9	7.8	0.2	0.68	0.90	0.70
GEI, Mcal	34.9	34.7	34.8	0.9	0.99	0.85	0.88
DEI, Mcal	20.6	21.1	20.3	0.5	0.50	0.32	0.82
Methane							
g/d	108.8	97.2	100.7	5.1	0.42	0.14	0.11
g/kg DMI	13.7	12.4	12.7	0.6	0.43	0.18	0.13
g/Mcal GEI	3.10	2.80	2.86	0.13	0.37	0.17	0.11
g/Mcal DEI	5.27[a]	4.62[b]	4.92[ab]	0.21	0.51	0.05	0.07
Carbon Dioxide							
g/d	5549[a]	5051[b]	5163[b]	172	0.19	0.05	0.02
g/kg DMI	701.8[a]	643.7[b]	660.0[ab]	18.1	0.27	0.06	0.03
CH_4:CO_2	0.020	0.019	0.019	0.001	0.67	0.70	0.56

[1]Linear effect on response variables

[2]Quadratic effect on response variables

[3]Biochar vs. No biochar inclusion

[a, b,] Means within a row with different superscripts are different ($P < 0.10$)